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FOREWORD

I am pleased to introduce the Proceedings of AGROSYM 2020. This year's edition of AGROSYM is the 11th one of the international symposium, but also the first of its kind as it was held virtually due to an uncertain "COVID-19" situation world-wide and in Bosnia and Herzegovina, which resulted in travel restrictions for most countries with no crisis end in sight.

The COVID-19 pandemic had multiple, far-reaching negative impacts on almost every aspect of our lives but it confirmed the centrality of science, knowledge and innovation in addressing the challenges facing humanity. In this context, the 11th Scientific International Agricultural Symposium "AGROSYM 2020" made an important contribution to the sustainable development of agriculture and food systems, in the Balkans and beyond, from environmental, social, economic and political points of view. We accepted around 550 contributions (abstracts and full papers) for presentation and publishing in the present Proceedings, which represent the results of cutting-edge international research from 70 countries worldwide. Plenary lectures focused on the CIVID-19 as well as the celebration of the International Year of Plant Health (IYPH). Indeed, keynotes addressed the impacts of the COVID-19 pandemic on agriculture, food systems and food security; the increasing risks and management challenges generated by invasive species in agriculture; and, last but not least, the role of plant health clinics in safeguarding global agriculture. Meanwhile, oral and poster presentations in parallel sessions addressed all AGROSYM thematic areas: 1) Plant production, 2) Plant protection and food safety, 3) Organic agriculture, 4) Environmental protection and natural resources management, 5) Animal husbandry, 6) Rural development and agro-economy, and 7) Forestry and agroforestry.

I do believe that the results reported in the present Proceedings will contribute to the dissemination of knowledge to the wider audience, practitioners and researchers in the fields of agriculture, food science, animal science, rural development, forestry and agroforestry. Indeed, research is of paramount importance to mitigate the impacts of the COVID-19 pandemic and foster transition towards agriculture and food systems that are more sustainable and resilient to crises and shocks.

Full-texts of the submitted contributions are available on AGROSYM website (http://agrosym.ues.rs.ba/index.php/en/archive). All papers included in the Proceedings were reviewed by international scholars.

Much appreciation is due to the authors of all papers, submitted and presented virtually, as well as to all symposium participants whose ideas and contributions provided for rich and lively discussion during the various sessions. Many thanks to all the authors, reviewers, session moderators and all colleagues for their help in editing the Proceedings. Special thanks go to the organizers, partners and sponsors for their unselfish collaboration and comprehensive support. Finally, special thanks to CIHEAM-Bari (Italy) for providing online platform and technical support for this year's virtual edition of AGROSYM.

Academician Dusan Kovacevic
Academy of Engineering Sciences of Serbia (AESS)
President of the Scientific Committee of
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PLANT PRODUCTION

FODDER PRODUCTIVITY OF A PERSPECTIVE VARIETY SUDANIAN GRASS CREATED BY THE VORONEZH STATE AGRARIAN UNIVERSITY

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Abstract

The article presents the results of breeding work to create promising varieties of Sudanese grass at the Voronezh State Agrarian University.
Annual forage grasses are of great importance in creating a solid fodder base for livestock. Sudan grass is characterized by high resistance to drought, and therefore is of great importance in the zone of unstable humidity. The culture has good fodder qualities and high productivity. In recent years a collection of sample varieties has been studied and parental forms with important economic and biological characteristics have been selected. As a result of the research a promising variety was selected, and it is planned to be transferred to the state variety test. In 2017-2019 a promising variety of Sudanese grass was tested in comparison with the zoned standard Voronezh 24 on forest-steppe in the Central Black Earth Region. It was noted that it is characterized by a more intensive development at the beginning of growth (it entered the bush formation phase faster than the control variety by 4 days, panicle formation – by 8 days), higher bushiness (7,4 pcs/plant with the Voronezh 24 - 6.0), by formation of thinner stems (stem diameter 0.7 mm) and long well-developed leaves, indicating a better quality of feed, as well as by a higher yield of green mass, which was 406 centners per hectare for two mowings, which is 36 centners per hectare higher than the standard. The sugar content in the green mass ranges from 11 to 11.9%.

Key words: *Sudan grass, variety, breeding material, productivity, green mass, seeds.*

Introduction

The implementation of the priority program for the development of animal husbandry adopted by the President and the Government of the Russian Federation is impossible without increasing the productivity of feed production by selecting the most productive crops and improving the technologies for their cultivation (Nikolaychenko, 2015). The most important link in creating a sustainable fodder base in the Central Black Earth Region is the cultivation of drought-resistant sorghum crops. The most promising of them is considered Sudanese grass - S. sudanense (Riper) Stapf.
In recent years, in the arid conditions of the Central Black Earth region, Sudan grass has been the most high-yielding of hay crops, it is characterized by increased bushiness, good regrowth after mowing or grazing and it can produce green mass throughout the summer and autumn (Pavlyuk, 2009). As for the main element of nutrition - protein, Sudanese grass among grasses is unparalleled. It retains this advantage both in the green mass and in the feed produced from it - hay, silage and grass meal. In the carotene content (60-65 mg / kg of green mass), Sudanese grass is not inferior to sorghum and almost doubles corn and oats (Bogatyrev, 1951). In arid weather conditions, it provides a stable yield compared to traditional forage crops, it is able to grow quickly after mowing, and can be used for silage, haylage, grass flour and green mass (Soloviev, 1955).
Young plants of Sudanese grass suffer from frosts of -2 ... −3 ° C, adults withstand −3 ... −4 ° C. Plants grow slowly in the first 3-4 weeks. The panicle formation phase is observed 40-45

days after emergence. Under favorable conditions, the height of the stem reaches a height of 2.5-3.5 m (Naumkin, 2017). Flowering occurs in July-August, seed ripening - in September-October. Seed yield can reach 3 t / ha. The crop of green mass reaches 35-40 t / ha.

In the system of green conveyors, Sudanese grass is sown in 2-3 terms with an interval of about 15 days. Finer feed gives condensed crops. Sudan grass is also grown in mixed crops with legumes (vetch, peas) and corn. The high sugar content in the feed mass contributes to its good silage.

Sudan grass is grown in relatively warm regions. It gives larger yields on fertile, well-moistened soils.

The best predecessors for Sudan grass are cereals and legumes, row crops. It belongs to the late sowing of agricultural crops (Torikov, 2019). The minimum temperature of the soil for seed germination is 8-10 ° C. The ordinary sowing method is ordinary with a seeding rate of 2.5-3 million pcs. per 1 ha or 25-35 kg / ha. Sowing in wide rows is possible with a decrease in the seeding rate to 14-18 kg / ha. When cultivating Sudan grass, it is necessary to combat soil crust and weeds (Shitikova, 2019). It is collected for green food until a panicle is formed at a height of 40-50 cm, with subsequent mowing - after 25-30 days, for the hay - when panicles appear. In favorable years, you can get up to three hayfields. It grows well when mowed or grazed on pastures (Pylnev, 2016).

Diversity in feed production, as in other sectors of agricultural production, plays a leading role in increasing the yield of fodder land, as well as in the collection of high-quality feed. The program for the selection of annual cereal grasses, including Sudanese grass, provides for the creation of high-quality varieties and hybrids of a new generation, adapted to the climatic conditions of a particular region, ensuring high and sustainable yields. Sudanese grass should have strong bushiness, branching of the stems, a tall and thin stalk with a dry and juicy kernel, it should grow vigorously after grazing or mowing green mass for hay and green fodder (Kryukova, 2020).

One of the main constraining factors for the widespread occurrence of Sudanese grass is its late maturity, therefore, the main purpose of the work is to select for a reduction in the growing season, combined with resistance to cold and rapid growth in the initial period. All this will allow promoting Sudanese grass in the more northern regions of the Russian Federation.

Material and methods

The studies were conducted in 2017-2019. fields of the Voronezh State Agrarian University, located in the forest-steppe zone of the Central Black Earth region. The climate of the zone is temperate continental. The soil of the experimental plot leached chernozem. Competitive variety testing were held in 2017-2019, was sown in four repetitions according to the methodology of the State test of agricultural crops (Methodology of state variety testing of agricultural crops, 1985).

Sowing was carried out in the first decade of May. The predecessor was oats, without fertilizer. Sowing ordinary, sowing rate of 2.5 million germinating grains per 1 ha. Variety Sudan grass Voronezh 17 was created in the Voronezh State Agrarian University by free pollination with subsequent mass selection from the hybrid population. As a standard, zonal Sudan grass of the Voronezh 24 variety was used.

Vegetation conditions during the years of research varied in temperature and the amount of precipitation. Thus, a variety of meteorological conditions in 2017-2019. allowed to conduct a comprehensive assessment of the studied forms of Sudan grass on the main economic and biological features and properties.

Results and Discussion

As a result of further study of the obtained hybrids, a varietal sample was selected combining high productivity with early maturity.

The results of the research to study the productivity of a promising variety of Sudan grass Voronezh 17 compared with the standard variety Voronezh 24, are presented in table 1.

Table 1. Seed quality for sowing the studied varietal samples, 2017-2019.

Varietal specimen	Voronezh 24	Voronezh 17
Mass of 1000 seeds, g.	14,1	14,9
Germination energy,%	82,5	84,3
Laboratory germination,%	89,5	89,0
Field germination,%	81,0	83,5

The weight of 1000 seeds in the new sample of the variety was on average 14.9 g in 3 years, and in the standard variety of Voronezh 24 - 14.1 g. We also noted that the germination energy and field germination was higher in the variety Voronezh 17.

In the field experiment, observations were made throughout the growing season (table 2).

Table 2. Duration of phases of development of Sudan grass, 2017-2019

Varietal specimen	Число дней от посева до				
	sprouting	tillering	panicle formation	mass flowering	full ripening of seeds
Voronezh 24	19	41	72	81	124
Voronezh 17	15	38	65	76	116

In the course of the research, we noted that the onset of the main phases of development in the Voronezh 17 is faster. A new varietal enters the panicle formation phase a week earlier than Voronezh 24, and therefore, both varieties can be used as part of a green conveyor. Voronezh 17 enters the full ripening phase 8 days earlier, which is a very important aspect of seed production of Sudan grass in the conditions of the Central Black Earth region, as ripening occurs in the second half of September, when rainfall can often interfere with harvesting.

Sudanese grass is cultivated both in wide-row crops and in a continuous ordinary way, each of which has its own advantages and disadvantages. So, with a continuous ordinary method of sowing, the green mass of Sudanese grass is thinner, tenderer and has many leaves, which increases its nutritional value and eatability.

The study of the structure of green mass by the elements of productivity (table 3). For this, 10 plants were consecutively selected from each plot and measurements were taken.

Table 3. The structure of the crop of green mass, 2017-2019

Varietal specimen	Plant height, cm	Stem diameter, cm	Bushiness, pcs.	The number of leaves	Sugar content in green mass, %	Largest sheet	
						length cm	width cm
first mowing							
Voronezh 24	180,0	0,75	6,0	6,8	11,2	45,6	3,1
Voronezh 17	177,9	0,70	7,4	7,0	11,9	49,6	2,7
second mowing							
Voronezh 24	145,8	0,49	5,4	5,6	11,0	49,4	2,5
Voronezh 17	146,2	0,36	7,0	6,1	11,0	54,6	2,3

From the data of table 3 we can conclude that the plants of the first mowing, Varieties of Voronezh 24 (180 cm), were the most prominent in height. But in the new variety specimen, a greater number of shoots on the plant was noted (7.4, in Voronezh 24 - 6.0). The thinnest stems were also in the variety specimen Voronezh 17, which indicates a higher quality of feed. In addition, it was noted that longer and narrower leaves formed in the new variety specimen. The sugar content in the green mass was slightly higher in the new variety.
Harvesting of green mass was carried out in the phase of panicle formation, and after regrowth of the green mass a second mowing was done (table 4).

Table 4. Crop of Sudan grass

Varietal specimen	Harvest of green mass of Sudan grass, centners from 1 hectare		In total for two mowing
	first mowing	second mowing	
Voronezh 24	196	174	370
Voronezh 17	223	183	406

From the data of the table it can be concluded that the highest yield was shown by the new variety specimen, the yield of which in total for 2 mowings was 406 kg / ha, and the control - 370 kg / ha.

Conclusion

As a result of the studies, it can be concluded that the new variety of Sudan grass Voronezh 17, in comparison with the standard variety, developed more intensively in the initial periods of growth (it entered the bush formation phase 4 days faster than the control variety; panicle formation was 8 days faster than the Voronezh 24 variety). The new variety showed higher bushiness (7.4, in Voronezh 24 - 6.0), thin stems (stem diameter 0.7 mm) and long leaves, indicating a higher quality of feed. The sugar content in the green mass in both varieties ranged from 11 to 11.9%. In addition, the studied sample of the variety had a higher yield of green mass, which was 406 centners per hectare for two mowings, which is 36 centners per hectare higher than that of the standard variety. In this connection, it is believed that its cultivation will bring greater productivity, and, accordingly, greater profit.

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THE EFFECT OF FERTILIZATION AND FRUIT ORDER ON STRAWBERRY FRUIT QUALITY

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Abstract

Fertilization is an important cultural practice in modern strawberry production, including the use of different types of fertilizer. The present research is aimed to evaluate the effect of fertilizer type and order in the truss on the fruit quality of strawberry cultivar 'Clery'. Fruit quality (mass, dimensions, shape index, firmness, soluble solids and total phenols) was monitored in three fertilizer treatments (biofertilizer, the combination of bio- and mineral fertilizer and mineral fertilizer) and four fruit positions in the truss (primary, secondary, tertiary and quaternary). Application of mineral fertilizer resulted in a significant increase in fruit firmness (10.4 N) while the use of biofertilizers containing bacteria of the genus *Bacillus* enabled fruits with significantly higher total phenolic content (276.6 mg GA/100 g fw) with the positive effect also exerted in secondary positioned fruits (486.7mg GA/100 g fw). Differences in quality traits were greater among different fruit positions, compared to fertilizer treatments although primary fruits were characterized by the highest values of mass and dimensions. Tertiary positioned fruits had the highest soluble solids content (11.7%) that did not significantly differ from the fourth batch of strawberry fruits (11.1%). Overall, the result of this study suggests that fruits at a lower position in the truss have better organoleptic composition while fruits on higher positions have better physical properties. Biofertilizers used in combination with mineral fertilizers have the potential to increase physical strawberry properties and the use of biofertilizers as supplements to mineral fertilizers can be considered an appropriate practice to ensure safe strawberry fruit production and help overcome environmental problems caused by the overuse of mineral fertilizers.

Keywords: *Strawberry, Biofertilizer, Mineral Fertilizers, Fruit order, Fruit Quality.*

Introduction

Strawberries are widely and highly consumed fresh and in processed forms, and their increasing production under glass makes them available all year round (Anttonen et al., 2006). Micronutrients and phenolic compounds concentration in berries, as well as commercial and organoleptic attributes, have been reported to change according to many pre-harvest conditions, such as genotype, environment, and cultivation techniques (Cordenunsi et al., 2002; Kafkas et al., 2007).

In modern strawberry production, fertilization is one of the most important cultural techniques because balanced fertilization has an important effect on both the fruit yield and fruit quality. An extensive body of research shows that the continuous use of mineral fertilizers leads to environmental contamination, with more than 50% of applied mineral fertilizers remaining unabsorbed, resulting in mineral loss, and thus posing a serious threat to the environment. Therefore, proper use and partial or complete substitution of mineral fertilizers with microbial inoculants i.e. biofertilizers can help overcome environmental problems caused by the overuse of mineral fertilizers. Also, food scientists study production and processing to develop new technologies that improve the quality and quantity of healthy food products, with the main

objective of increasing food production without affecting food quality and environment, while fulfilling consumer expectations (González-Aguilar et al., 2010).
The flowers of the strawberry are collected in cymose inflorescence called dicasia. Dicasia consists of one primary, two secondary, four tertiary, and eight quaternary flowers that open according to the time of origin. Despite changes in strawberry fruit phytochemical content during ripening (Kosar et al., 2004; Ferreyra et al., 2007), many published studies employ a commercial type of picking (by color) to determine harvest date and ignore developmental time, truss position and the effect these parameters have on fruit chemical composition (Tsormpatsidis et al., 2011). Thus, this study aimed to evaluate the potential of fertilizers as well as fruit order on the quality of the strawberry fruit.

Materials and methods

Experimental layout and orchard management

The field experiment was carried out in the strawberry plantation at the Fruit Research Institute, Čačak, located in central Čačak (43º 54' N latitude, 20º 21' E longitude, 242 m altitude), in the Western Morava valley (Western Serbia). Strawberries were planted in August 2015 in double rows on beds covered with black polyethylene foil. Plant spacing was 30×30cm, and spacing between beds was 40 cm. 'Clery', one of the predominant strawberry cultivars in the Republic of Serbia, was selected for this study in the first year after planting (2016). The layout of the experiment was a completely randomized design, with the effect of two factors, viz. fertilizer and fruit order analyzed. In addition to standard cultivation practices, the plants were regularly irrigated according to soil humidity. The fertilizers were applied through fertigation.
The experimental procedure included three fertilization treatments: B, the bacterial inoculum of *Bacillus* sp. ('Bacilomix'); B+M, half of the standard recommendations mineral fertilizers and bacterial inoculum of *Bacillus* sp.; M, mineral fertilizers with different formulation ratios according to standard recommendations for strawberry production. Microbiological fertilization with a bacterial inoculum of *Bacillus* sp. involved fertigation with 10–12 L/ha of the inoculum three times per month during the growing season. The bacterial titer in the inoculum was $20–40\times10^6$ cm^3. Mineral fertilizers were applied according to the phenological stage of the plant, as follows: at the beginning of the growing season, starter fertilizer NPK Poly-Feed Drip 11-44-11 with micronutrients; during intensive plant growth and flower bud emergence, two applications of NPK Poly-Feed Drip 20-20-20 with micronutrients at a seven-day interval; during flowering, fruit set, growth, and ripening, five applications of the complex mineral fertilizer NPK Poly-Feed Drip 16-8-32+2MgO at ten-day intervals; during intensive fruit growth and ripening, in addition to the former formulation, two applications of Multi-Cal (15.5% N and 26.5% CaO) and Multi-KMg (12% N; 43% K and 2% MgO) at a 10-day interval. Combined application of microbiological and mineral fertilizers consisted of a half amount of aforementioned microbiological and mineral fertilizers each. The treatments were represented by 60 plants each (3 replicates of 20 plants).
The strawberries were harvested at commercial maturity stage. Samples for analysis were taken during the whole harvest and consisted of primary (I), secondary (II), tertiary (III) and quaternary fruits (IV) (sixty strawberries from each order of fruit, 20 fruits per 3 replications). Fresh fruits were used for initial physicochemical measurements and then the samples were stored at -20°C till analysis of total phenolic content (TPC).

Determination of fruit quality

Twenty fruits in each replication were randomly selected to determine average fruit weight using the 'Metler' balance (±0.01 g accuracy) and the data were expressed in g per fruit. The fruit dimensions (length and width) were measured by a digital moving scale (Carl Roth,

Germany) with an accuracy of ± 0.05 mm. The index of the fruit shape was obtained by calculation, determining the ratio of the length and width of the fruit. The firmness of the fruit was determined by a penetrometer, and the values were expressed in N. Soluble solids (SS) was determined using a hand refractometer (Pocket PAL-1, Atago, Japan), and values were expressed in %.

Total phenolic content was measured using a modified Folin-Ciocalteu assay (Liu et al., 2002). Samples weighing 4 g of fresh strawberries were blended in a food processor for 1 minute with 40 ml of 80% of aqueous methanol. The well-blended solution was centrifuged at 10 000 g for 15 minutes at 20°C, and the supernatant was separated. Shortly, 40 µL of fruit extracts or gallic acid standard solution were mixed with 3.16 mL of distilled water. In the next phase, 200 µL of Folin-Ciocalteu reagent was added and allowed to stand for 8 minutes before adding 600 µL of 20% sodium carbonate solution. The solution was well mixed and absorbance of the samples and standards were measured spectrophotometrically (Jenway 6300, UK) at 765 nm. TPC was calculated as mg of gallic acid equivalents per 100 g fresh weight of the sample (mg GAE/ 100 g FW). The total phenolic concentration was derived as a function of the equivalent absorbance of gallic acid in the range 50 to 500 mg/L (R^2=0.99).

Results and discussion

Fruit size is one of the most important traits in the selection of new genotypes (Di Vittori et al., 2018). Describing the characteristics of strawberry cultivars grown in northern Italy, Lucchi et al. (2015) point out that the regular conical shape of cultivar 'Clery' is its good characteristic, while the tendency to reduce the dimensions of the fruit during the second part of the harvest is a bad characteristic of this cultivar. Similar results were obtained in our study where the dimensions of the fruit in strawberry cultivar 'Clery' on third and tertiary positioned were lower. In the research of Milivojević et al. (2015), the fruit weight of the cultivar 'Clery' (22.8 g) grown in the open field was close to the fruit weight recorded in a previous study by the same authors (Milivojević et al., 2009) in the cultivar 'Clery' cultivated in greenhouse conditions (22.9 g). However, the fruit weight of the cultivar 'Clery' in our studies was lower (from 20.9 in mineral to 21.4 g in microbiological fertilizer treatment) compared to the above values.

A fertilization regime was found to influence the fruit nutritional quality of cultivated strawberry (Di Vittori et al., 2018). The data presented in our study show that fertilizers did not affect most of the fruit quality parameters, except in firmness and total phenols (Table 1). The high fruit firmness obtained in mineral fertilizer treatment may be related to the application of fertilizers with high calcium content. The obtained results are in agreement with those of Treder (2004) and Wójcik and Lewandowski (2003), who point out that the basic element responsible for fruit firmness is calcium.

According to Jouquand (2008), the sensory quality of strawberry fruit is the result of complex balance among sweetness, aroma, texture and fruit appearance. The same authors point out that genotypes assessed as "not sweet" also had low soluble solids content and this parameter is generally a good indicator of consumer acceptability./for the acceptability of consumers. The average values of soluble solids content as a function of the fertilizer types and fruit order ranged from 9.7 to 11.7%. Soluble solids content did not vary significantly among the tested fertilizers while the position of the fruit significantly influenced these properties. Tertiary fruits had the highest soluble solids content (11,7%) that did not differ significantly from the soluble solids content of the fourth-order strawberry fruits (11,1%).

Table 1. Fruit quality of strawberry cultivar 'Clery' depending on fertilizer and fruit order.

		Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Shape index	Firmness (N)	Soluble solids (%)	Total phenols (mg GAE/100 g FW)
Fertilizer (A)	B	21.4±4.5 a	39.6±3.0 a	34.3±2.2 a	1.1±0.0 a	7.3±0.9 b	10.3±0.4 a	276.6±38.4 a
	B + M	21.3±3.9 a	39.9±2.7 a	33.4±2.0 a	1.2±0.0 a	8.5±1.0 ab	10.5±0.3 a	234.3±15.7 b
	M	20.9±3.9 a	39.6±2.9 a	33.3±2.2 a	1.2±0.0 a	10.4±1.1 a	11.1±0.5 a	204.6±11.5 c
Fruit order (B)	I	41.6±2.6 a	52.3±1.1 a	43.8±1.3 a	1.2±0.0 a	7.3±0.5 a	10.0±0.4 bc	227.1±11.3 b
	II	22.3±0.8 b	42.4±0.4 b	35.0±0.5 b	1.2±0.0 a	9.0±1.0 a	9.7±0.2 c	283.7±51.0 a
	III	13.2±0.5 c	37.3±0.9 c	30.5±0.6 c	1.2±0.1 a	8.4±1.8 a	11.7±0.6 a	206.6±25.4 c
	IV	7.8±0.4 d	26.8±0.5 d	25.4±0.6 d	1.1±0.0 b	10.2±1.3 a	11.1±0.4 ab	236.7±7.5 b
A		ns	ns	ns	ns	*	ns	*
B		*	*	*	*	ns	*	*
A×B		ns	ns	ns	ns	ns	ns	*

Data represent the means of three replicates±standard error. The different lower-case letters in the columns indicate statistically significant differences among the mean values relative to fertilizer and fruit position at P≤ 0.05 level (Duncan's test).

Recently, also the nutritional value of berries, as the content of bioactive compounds with healthy effects on the final consumer, is being considered to better characterize the fruit quality (Di Vittori et al., 2018). Strawberry fruits, compared to other fruit species, contain high amounts of phenolic compounds, which have shown a positive effect against free radicals in *in vitro* tests (Aaby et al., 2007; Seeram et al., 2009). The phenolic components in berries have many different biological functions, including a role in plant growth, development, and protection (Nile and Park, 2014). They play a role in pigmentation, have antimicrobial and antifungal functions, provide protection against insects and ultraviolet radiation, chelate toxic heavy metals and defend against free radicals formed in the process of photosynthesis (Beer et al., 2004; Parry et al., 2005). In regard to the content of total phenols in our study, the influence of the fertilizer treatment was significant. Co-inoculation of strawberry plants showed better results in concentration of the total phenols compared to those recorded in plants cultivated following the standard fertilization protocol. Microbial biostimulants can improve root activity, increase water and nutrient uptake, and enhance crop tolerance to environmental stresses, yield and product quality, which may explain the high phenolic content in microbial inoculation treatment in our study. It is also known that beneficial soil microorganisms, like bacteria and arbuscular mycorrhizal (AM) fungi can influence the plant secondary metabolic pathways (Zeng et al., 2013).

Anttonen et al. (2006) found that fruit order significantly affected the phenolic content in fruits. The same authors stated that the levels of the total phenolics, ellagic acid, and antioxidant activity were found to increase from primary to tertiary fruits. Fruit order had a significant effect on the content of total phenols in our study. Second-order fruits had a significantly higher content of total phenols compared to other position of fruits. However, it is important to point out that the highest content of the aforementioned parameter was recorded in interaction between secondary fruit and microbiological fertilizer (Figure 1).

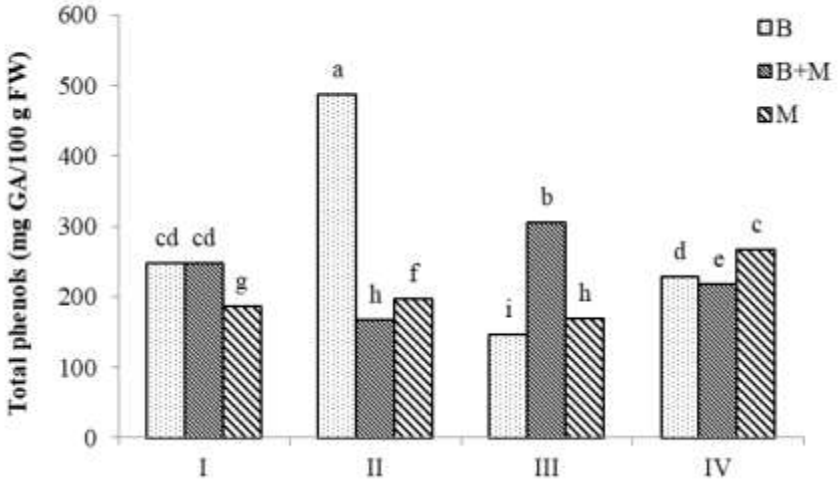


Figure 1. Total phenols in the fruit of strawberry cultivar 'Clery'. The different small letters at the top of columns indicate significant differences in total phenols at $P \leq 0.05$ by Duncan's test.

Principal components analysis (PCA) is a way of identifying the patterns in the data and expressing the data in such a way as to highlight their similarities and differences. In this study, the PCA analysis was applied to the complete data set (mass, dimensions, firmness, SS, TPC) to explain the correlations between the combination fertilizer/fruit order and to identify group patterns (Figure 2). The first group was composed of fruits with a high fruit mass and dimensions. This group includes primary and secondary fruits in mineral and a combination of mineral and microbiological fertilizer. Tertiary and quaternary fruits in combination with mineral and microbiological fertilizer belong to a group of fruits with good firmness and soluble solids content. The PCA analysis confirmed that the differences, which occurred in the physical and chemical composition of fruit, depended on the fruit position more than fertilizer type. Based on the presented data, it can be concluded that specific fruit properties depend on developmental time which is important for their potential commercial (fresh) or industrial use.

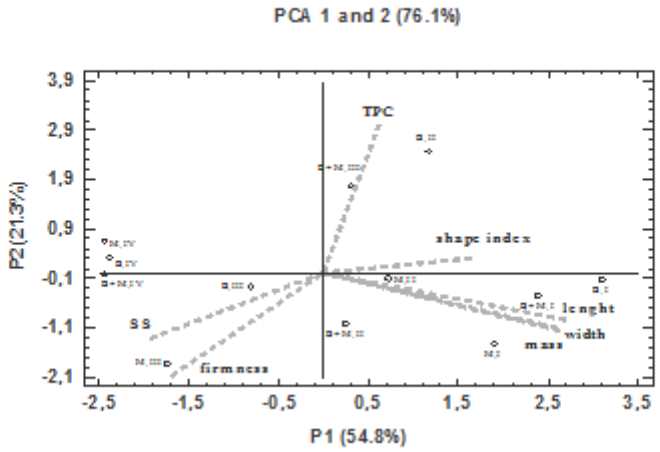


Figure 2. Biplot based on principal components analysis (PCA) for fruit quality properties in strawberry cultivar 'Clery'.

Conclusion

Bearing in mind that there was no difference in the fruit quality in combined use of mineral and bio fertilizers compared to the treatment with mineral fertilizers, fertilization modifications may provide a potential means to increase the fruit quality (weight, dimensions and firmness) as well as health-related value of strawberry by altering the phenolic contents of the fruit. Choosing cultivar as well as fruit order can affect the quality of fresh fruits and strawberry end products.

Acknowledgments

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MARKER ASSISTED SELECTION FOR β-CAROTENE RICH MAIZE: TWO-LEVEL SELECTION PROCEDURE IN BC_2 GENERATION

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Abstract

This paper is a part of the breeding program conducted at the Maize Research Institute "Zemun Polje" (MRI), with the aim to convert the standard maize to β-carotene rich genotypes adapted to temperate regions using the marker assisted selection. When SSR marker specific to the *crtRB1* gene has been tested on parental lines, distinct polymorphism was observed between the donor and recurrent parents. The same marker has also been validated in BC_1 generation when heterozygous individuals were clearly distinguishable from the homozygous dominants. The results presented herein refer to two-level selection procedure in BC_2 generation. First, BC_2 plants were analyzed with *crtRB1*-specific molecular marker to separate heterozygotes from dominant homozygotes. Percentage of heterozygous plants was approximately 50%, which was in accordance with the expected Mendelian ratio of 1:1. Second, the selected heterozygotes were screened with 30 polymorphic SSR markers distributed throughout the maize genome to identify genotypes with the highest recovery of recurrent parent's genome (RPG). The RPG values among three analyzed parental lines and their respective progenies ranged from 85-99%. For each line separately, RPG values were: 86-97% (RP_1), 90-95% (RP_2) and 85-99% (RP_3). Plants with RPG above 95% were selfed to produce BC_2F_2 generation in which homozygous recessive individuals would be identified. Those genotypes will be subjected to biochemical and phenotypic evaluation to confirm their nutritional and agronomical superiority. Finally, these β-carotene enriched lines, as well as the resulting improved hybrids, can be used in the biofortification programs.

Keywords: *β-carotene, crtRB1 gene, maize, marker assisted selection*.

Introduction

Vitamin A deficiency (VAD) is a public health problem and the leading cause of preventable childhood blindness, anaemia and weakened host resistance to infection, which can increase the severity of infectious diseases and risk of death (WHO, 2009). Since vitamin A cannot be synthesized inside the human body and it needs to be provided through diet, breeding for increasing provitamin A (ProVa) carotenoids in staple crops would be a viable strategy to minimize the adverse effects of VAD (Bouis *et al.*, 2011; Muthusamy *et al.*, 2015).

One of the most important breeding objective is improving the ProVA content of maize grain. Two genes, *lcyE* on chromosome 8 and *crtRB1* on chromosome 10, have the most significant effect on ProVA concentrations in maize grain. According to Babu *et al.* (2013), *crtRB1* gene had a much larger effect on ProVA concentration than *lcyE*. Three polymorphisms were detected in *crtRB1* that were significantly associated with the β-carotene concentration in maize kernels (Yan *et al.*, 2010), of which *crtRB1* 3′TE favourable allele alone caused two to tenfold variations in the β-carotene concentration among the inbreds (Babu *et al.*, 2013; Muthusamy *et al.*, 2014). The 3′TE (transposable element) polymorphism of the gene that spans the 6th exon and the 3′-UTR (untranslatedregion) generates three allele sassociated with

altering β-carotene accumulation: *allele 1* (543 bp; without TE insertion), *allele 2* (296 bp+875 bp; with 325 bp TE insertion) and *allele 3* (296 bp+1221 bp+1880 bp; with 1250 bp TE insertion). *Allele 1* of the *crtRB1* gene is favourable and increases the level of β-carotene, whereas *allele 2* and *allele 3* cause unfavourable effects (Yan *et al.* 2010).

The accelerated development of β-carotene enriched maize using marker assisted selection (MAS) has been reported by Muthusamy *et al.*, (2014). As concluded by these authors, this breeding strategy holds immense promise as it precisely selects desirable plants and eliminates large scale biochemical estimation in the segregating generations. These β-carotene enriched lines, as well as the resulting improved hybrids, can be used in the biofortification programs alleviating VAD worldwide.

This paper is a part of the breeding program conducted at the Maize Research Institute "Zemun Polje" (MRI) with the aim to convert the standard maize to β-carotene rich genotypes adapted to temperate regions using the marker assisted selection. When SSR marker specific to the *crtRB1* gene has been tested on parental lines, distinct polymorphism was observed between the donor and recurrent parents (Kostadinović *et al.*, 2018). The same marker also clearly distinguished the heterozygous individuals from the homozygous dominants in BC_1 generation. The main objective of this research was the two-level selection procedure in BC_2 generation. First, BC_2 plants were analyzed with *crtRB1*-specific molecular marker to separate heterozygotes from dominant homozygotes. Second, the selected heterozygotes were screened with a set of polymorphic SSR markers distributed throughout the maize genome to identify genotypes with the highest recovery of recurrent parent's genome (RPG).

Material and Methods

Plant material

Three MRI commercial inbred lines adapted to the local environmental conditions in Serbia were used as the recurrent parents (RP_1, RP_2 and RP_3). Due to their excellent combining abilities, these lines are components of the leading MRI hybrids. As a donor of the favourable allele of *crtRB1* gene, i.e. β-carotene increased content, a line provided by the International Maize and Wheat Improvement Center (CIMMYT) was used.

The conversion process is given in Figure 1. F_1 plants were backcrossed onto reccurent parent line to generate BC_1 progeny. The BC_1 plants that were heterozygous for the *crtRB1* locus were selected for backcrossing. The results of this paper relate to the two-level selection procedure carried out in BC_2 generation (emphasized in Figure 1). First, BC_2 plants were analyzed with *crtRB1*-specific molecular marker to separate heterozygotes from dominant homozygotes. Second, the selected heterozygotes were screened with 30 polymorphic SSR markers distributed throughout the maize genome to identify genotypes with the highest recovery of recurrent parent's genome (RPG). Those plants were selfed to produce BC_2F_2 seeds.

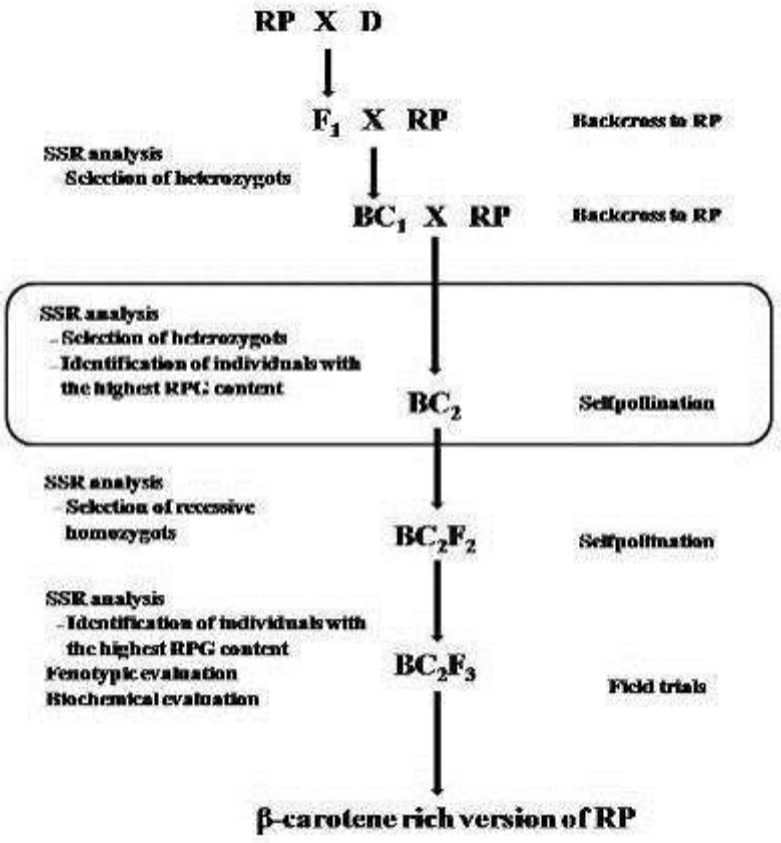


Figure 1. Schematic presentation of MAS for conversion of standard maize to β-carotene rich maize line (RP-recurrent parent, D-donor line, RPG-recurrent parent's genome)

DNA extraction

Genomic DNA was isolated from the four-weeks-old plants applying modified Dorokhov and Klocke protocol (1997). The DNA was quantified using biospectrometer (BioSpetrometer kinetic, Eppendorf) and diluted to a working concentration of 20 ng/μL.

Foreground selection

The set of primers presented in Table 1. has already shown codominant polymorphism between the donor and recurrent parents (Kostadinović *et al.*, 2018). Therefore, it was used in foreground selection for the *crtRB1* gene.

Table 1. The set of primers used as a foreground selection marker for the *crtRB1* gene

Primer	Sequence
crtRB1-3′TE-F	5′-ACACCACATGGACAAGTTCG-3′
crtRB1-3′TE-R1	5′-ACACTCTGGCCCATGAACAC-3′
crtRB1-3′TE-R2	5′-ACAGCAATACAGGGGACCAG-3′

Polymerase chain reaction was carried out in 20 μL reaction volume containing: DreamTaq™ Green PCR Master Mix (2X) (Thermo Scientific™, USA), 0.25 μM primers and 20 ng DNA template.

Background selection

Simple sequence repeat (SSR) analysis was done with 30 polymorphic SSR markers spanning over the whole genome, selected from the maize database (www.maizegdb.org) (Table 2). PCR was carried out in 25 μL reaction volume containing: DreamTaq™ Green PCR Master Mix (2X) (Thermo Scientific™, USA), 0.5 μM primers and 20 ng DNA template.

Table 2. The list of SSR markers used in background selection

	Name	Bin	Repeat	Sequence (forward and reverse)
1	umc1282	1.00	(AT)6	5'-TACACTACACGACTCCCAACAGGA-3'
				5'-GCGAGGGTTCTTTCCATAGAGAAT-3'
2	umc2230	1.05	(AGC)5	5'-ACGCGACGACTTCCACAAG-3'
				5'-ACACGTAATGTCCCTACGGTCG-3'
3	umc2047	1.09	(GACT)4	5'-GACAGACATTCCTCGCTACCTGAT-3'
				5'-CTGCTAGCTACCAAACATTCCGAT-3'
4	umc2129	2.07	(CGC)5	5'-ACGTGGTCATCACTCACCGC-3'
				5'-AAGGAGGAGCGTTCTCGTGG-3'
5	umc1265	2.02	(TCAC)4	5'-GCCTAGTCGCCTACCCTACCAAT-3'
				5'-TGTGTTCTTGATTGGGTGAGACAT-3'
6	umc1535	2.05	(AT)7	5'-GGCAGAGAGATGAAAAAGAATGGA-3'
				5'-CAAGGCACCCACACACATACATA-3'
7	bnlg1456	3.05	AG(15)	5'-CTCTAGGTGGTTAAGATTAACTCATT-3'
				5'-TTCATGAGGACCGTGTTGAA-3'
8	umc1273	3.08	(AAG)4	5'-GTTCGCTGCTGCTTCTTATATGCT-3'
				5'-AATTGGCGCAGGCTATAGACATTT-3'
9	bnlg1257	3.09	AG(28)	5'-CGGACGATCTTATGCAAACA -3'
				5'-ACGGTCTGCGACAGGATATT-3'
10	umc1418	4.08	(GGAAG)4	5'-TCACACACACACTACACTCGCAAT-3'
				5'-GAGCCAAGAGCCAGAGCAAAG-3'
11	bnlg1784	4.07	AG(13)	5'-GCAACGATCTGTCAGACGAA-3'
				5'-TTGGCATTGGTAATGGGTCT-3'
12	umc2360	4.08	(GCC)4	5'-TAGCAGCTAGCTTCAGTCACAGGC-3'
				5'-CAGATCGGACTACTGGTGGCTAAG-3'
13	bnlg1046	5.03	AG(39)	5'-TGAGCCGAAGCTAACCTCTC-3'
				5'-GATGCAAAGGAGGTTCAGGA-3'
14	umc2373	5.04	(GCT)4	5'-ACCCAAGTGAGGTGAAGTGAAGC-3'
				5'-TATGGTACAGGCACAGCAGCAGTA-3'
15	umc1792	5.08	(CGG)5	5'-CATGGGACAGCAAGAGACACAG-3'
				5'-ACCTTCATCACCTGCAACTACGAC-3'
16	umc1006	6.02	(GA)19	5'-AATCGCTTACTTGTAACCCACTTG-3'
				5'-AGTTTCCGAGCTGCTTTCTCT-3'
17	umc1887	6.03	(CGA)4	5'-CTTGCCATTTTAATTTGGACGTTT-3'
				5'-CGAAGTTGCCCAAATAGCTACAGT-3'
18	umc2375	6.06	(GCG)4	5'-GCCGTACTGATGTGATGGTCC-3'
				5'-TCTGACATTGTCCTCTTGACCAAA-3'
19	umc1324	7.03	(AGC)5	5'-ATCCATCATCATCATCATTGCTTG-3'
				5'-ATGTCATCATGTACCAGGTGTTGG-3'
20	umc1695	7.00	(CA)8	5'-CAGGTAATAACGACGCAGCAGAA-3'
				5'-GTCCTAGGTTACATGCGTTGCTCT-3'

21	umc1782	7.04	(GAC)4	5'-CGTCAACTACCTGGCGAAGAA-3' 5'-TCGCATACCATGATCACTAGCTTC-3'
22	bnlg1782	8.05	AG(13)	5'-CGATGCTCCGCTAGGAATAG -3' 5'-TGTGTTGGAAATTGACCCAA-3'
23	phi080	8.08	AGGAG	5'-CACCCGATGCAACTTGCGTAGA-3' 5'-TCGTCACGTTCCACGACATCAC-3'
24	umc2355	8.03	(CCT)5	5'-CTACTCCCCGAAGCCGTCTAAG-3' 5'-CGGGTTGTTGTTGGAGTAGGAC-3'
25	umc2393	9.00	(ACG)7	5'-CAACTCGATCCAGACCACACATAG-3' 5'-CTCTTGGTTGTTTGTTTCCTTGCT-3'
26	umc1040	9.01	(CT)11	5'-CATTCACTCTCTTGCCAACTTGA-3' 5'-AGTAAGAGTGGGATATTCTGGGAGTT-3'
27	umc1492	9.04	(GCT)4	5'-GAGACCCAACCAAAACTAATAATCTCTT-3' 5'-CTGCTGCAGACCATTTGAAATAAC-3'
28	umc1827	10.04	(GAC)6	5'-GCAAGTCAGGGAGTCCAAGAGAG-3' 5'-CCACCTCACAGGTGTTCTACGAC-3'
29	umc1506	10.05	(AACA)4	5'-AAAAGAAACATGTTCAGTCGAGCG-3' 5'-ATAAAGGTTGGCAAAACGTAGCCT-3'
30	bnlg1839	10.07	(AG)24	5'-AGCAGACGGAGGAAACAAGA-3' 5'-TCTCCCTCTCCCTCTTGACA-3'

The PCR and polyacrylamide gel electrophoresis, both in foreground and background selection, were performed as explained in Kostadinović *et al.*, (2018). In foreground selection, the size of the amplification products was determined comparing with the 100 bp molecular weight ladder. In background selection, SSR profiles were converted into a binary matrix based on the presence (1) or the absence (0) of a specific allele. Genetic similarity (GS) was calculated in accordance with Dice (1945): GSij = 2a/2a+b+c; where: a is the number of fragments present in both variety i and j (1,1), b is the number of fragments present in i and absent in j (1,0), c is the number of fragments absent in i and present in j (0,1). Marker data analyses were performed using statistical NTSYSpc2 program package (Rohlf, 2000).

Results and Discussion

The two-level marker assisted selection in BC_2 generation implied the application of molecular markers both for precise transfer of genomic regions of interest (foreground selection) and the recovery of the recurrent parent's genome (background selection). The first goal was identification of heterozygous plants with molecular marker specific for the *crtRB1* gene. Out of 180 analyzed plants (60 plants per line), 87 (48%) were identified as heterozygous and 93 (52%) as dominant homozygous, which was in accordance with the expected Mendelian ratio of 1:1 in backcross generations. Out of these 87 heterozygous individuals, 30 originated from RP_1, 28 from RP_2 and 29 from RP_3. Figure 2 shows the codominant nature of *crtRB1*-3′TE marker that can distinguish homozygous and heterozygous individuals. Lanes 3, 4, 5, 6, 7, 12 and 13 were heterozygous and lanes 8, 9, 10, 11 and 14 were dominant homozygous individuals.

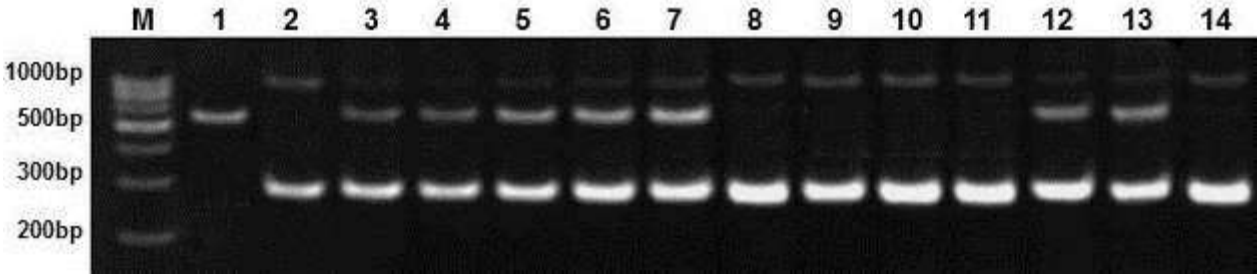


Figure 2. SSR profile of BC_2 individual plants detected with the *crtRB1*-specific marker. M:100bp DNA ladder, 1-donor parent, 2: recurrent parent, 3-14: BC_2 individuals.

Once the heterozygotes were identified, our second goal was to determine genetic similarity among these individuals and their recurrent parents with SSR markers distributed over the maize genome. The 30 SSRs was chosen for this analisys based on previously shown polymorphism (Kostadinovic *et al.*, 2018), since monomorphic markers that are not able to distinguish the two parental genotypes bear no value in selection work (Miah *et al.*, 2015). Total number of alleles detected with 30 informative markers was 44 for RP_1, 43 for RP_2 and 50 for RP_3, average being 1.47, 1.54 and 1.72, respectively. These results are similar to those previously reported in maize inbreds. In the study by Kanagarasu *et al.* (2013), the average number of alleles was 2.3, in Lopes *et al.* (2014) 2.6, while in Kostadinović *et al.* (2018) 2.5. Somewhat higher values in these studies can be explained by the higher number of analyzed genotypes and their diversity, as well as the number of markers used in the analysis (Mehta *et al.*, 2017).

Genetic similarity values between three analyzed parental lines and their corresponding BC_2 progenies, calculated using Dice coefficient, ranged from 0.85 to 0.99 (85-99% RPG). As explained in Muthusamy *et al.*, 2014, the variable proportion of RPG among BC_2 progenies is due to fixation of different proportion of recurrent parent alleles among the heterozygous plants. For each line separately, RPG values were: 86-97% (RP_1), 90-95% (RP_2) and 85-99% (RP_3). Average values for the RPG content ranged from 92 to 94%. Similar results have been reported in Muthusamy *et al.*, (2014), where RPG values ranged from 83.1% to 93.7%. Babu *et al.*, (2005), got the average RPG of 78.4% and they selected three plants with the highest values (93-96%) for developing the next generation. Thakur *et al.*, (2014) reported average RPG values from 90.1-97.2%, while Singh and Ram (2014) reported 82.5-98.5%.

In our study, a great acceleration of recipient genome recovery was achieved. Theoretically, the proportion of the RPG after *n* generations of backcrossing is given by $(2^{n+1} - 1)/2^{n+1}$ (Collard *et al.*, 2005). For BC_2 generation, that value should be 87.5%. In our case, 80% of progenies had RPG above this theoretical value. Another significant accomplishment were progenies with 99% of the RPG, the value theoretically achieved in BC_6 generation. These results confirmed that the combined approach of phenotypic and marker assisted selection reduces the time and economize the resources involved in the development of the favourable genotypes.

Plants with the highest RPG were self-pollinated to produce BC_2F_2 generation which will be subjected to the foreground selection before flowering to identify homozygous recessive individuals. Those genotypes will be subjected to biochemical and phenotypic evaluation to confirm their nutritional and agronomical superiority. Finally, these β-carotene enriched lines, as well as the resulting improved hybrids, can be used in the biofortification programs.

Conclusions

Being the codominant marker, *crtRB1*-3′TE clearly distinguished heterozygous individuals

from the homozygous dominants. That enables its utility in foreground selection for the *crtRB1*gene in our MAS program. Selected polymorphic SSR markers were employed in background selection to detect the recovery of the recurrent parent's genome. The RPG content of the BC_2 generation was between 85-99%. Progenies with the highest RPG values were selfed to produce BC_2F_2 generation for marker assisted introgression of the favourable allele of *crtRB1* into the local maize genotype. This study confirmed the efficiency of molecular markers both for foreground and background selection.

Acknowledgement

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IMPACT OF FOLIAR APPLICATION 'STOPIT' ON FRUIT QUALITY AND STORAGE OF 'GRANNY SMITH' APPLE (*Malus* × *domestica* Borkh.)

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Abstract

The paper presents the two-year results (2018–2019) of foliar product 'Stopit' application on pomological traits and storage of the apple cultivar 'Granny Smith'. Foliar treatments in apple orchards were performed four times (on June 7th, July 2nd, July 20th, August 15th – in 2018; June 14th, July 5th, July 26th, August 16th – in 2019) during vegetation, that is, from the beginning of June until middle of August. At harvest as well as after storage of fruits (120 days) in a cooler at normal atmospheric pressure, fruit morphometric traits, firmness and soluble solids content of the cultivar 'Granny Smith' and its control were studied comparatively. Analysis of the obtained results pointed to a statistically significant impact of storage period (except the soluble solids content), genotype (except fruit width) and agroecological conditions in the period of study as well as interaction effect of all variability factors on tested parameters. Values of the fruit mass ranged from 157.28 to 202.56 g, width from 60.11 to 71.72 mm, height from 71.30 to 80.03 mm and the content of soluble solids from 11.13 to 14.41 °Brix. Application of foliar product 'Stopit' containing calcium (Ca) in the form of calcium chloride ($CaCl_2$) conditioned a more favorable effect on pomological traits of the control variant of cv 'Granny Smith', whereas during the study period, higher values were found in the first year. From the economic perspective of apple production, values of the fruit firmness as one of the most important parameters of fruit quality were lower after taking the fruits out of the cooler although the values in each variant were higher compared to control.

Keywords: *Apple, Storage, Fruit quality.*

Introduction

By the volume of production, apple is the world's most important temperate fruit species taking the fourth place right after citrus fruits, grapes and bananas, with the total production of 86.142.197 t. In the Republic of Serbia, apple is important pome fruit species which is grown under area of 25.917 ha, with a total production of 460.404 t (Faostat, 2018). Apple fruits can be consumed fresh immediately after harvest or after storage (Folta and Gardiner, 2009). Good market supply with most diverse apple fruits demands a manufacturer's offer of high external and internal quality (O'Rourke, 2003). In the past decades, one of the more commercially important and wanted apple species on the market is cv 'Granny Smith'. The respective cultivar is characterized by the long vegetation period, late ripe, long storage period where later distribution on the market is possible (Milošević, 1997). Sams et al. (2008) quote that many physiological and pathological disturbances of apple fruits are conditioned by the content of calcium (Ca^{2+}) in the fruit tissue. Concentration of calcium in plant tissue has an extremely valuable role in maintaining the quality of fruits after harvest. Numerous authors (Hossain et al., 2005; Misra and Gupta, 2006; Naeem et al., 2009) emphasize that application of the calcium based composition have positive effects on cell membrane stabilization and the fruit age delay. It maintains the fruit firmness, decreases frequency of the so called 'bitter pit'

and internal decay of the fruit (Raese and Drake, 2002; Dierend and Rieken, 2007; Suljevic et al., 2011). Considering that, in the lack of calcium, all aging processes in fruits flow much faster and the fruits have lower storage capacity. There are numerous products on the market containing calcium that can be used before and after harvest, delaying fruit senescence with no harmful effect on the consumers (Lester and Grusak, 1999). One of the products of greater importance with which we can prevent or remedy the lack of calcium, and therefore to affect apple fruit quality is 'Stopit'.

The aim of the research is to determine the impact of foliar application 'Stopit' on the fruit quality and storage of 'Granny Smith' apple in a cooler at normal atmospheric pressure.

Material and Methods

The examinations were conducted in the period from 2018 to 2019 in the production-experimental apple orchard on the site of the Fruit Research Institute in Čačak (43° 89' 40" and 20° 43' 42" IGD, 233 m above sea level) in Serbia. The orchard was set up in 2006 and scions were grafted on M rootstock. Training system applied was palmeta. Planting distance was 4 × 1.25 m (2.000 trees ha $^{-1}$). During the examinations, standard agro-technical and plantation maintenance measures were applied.

The research included the foliar application of 'Stopit' based on calcium chloride, with high concentration of calcium (224 g/l). Application time of this product includes the interval from the beginning of flowering until the end of fruit maturation phenophase, i.e. the phase of pigmentation until harvest, in the amount of 5–10 L ha^{-1}. In that regard, foliar application of 'Stopit' was carried out on 20 trees in four replications (a total of 80 trees per treatment), from the beginning of June until the mid of August, each 21 day. 'Stopit' was used in the amount of 7,5 L ha^{-1} (150 mL on 10 L of water). In 2018, the treatment was carried out on June 7th, II was on July 2nd, III on July 20th and IV on August 15th. In 2019, treatments were performed as follows; I on June 14th, II – July 5th, III July 26th , IV – August 16th. Foliar application was carried out using the motor sprinkle SR 420 (STIHL International GmbH Waiblingen, Germany), with consumption of 1.000 L ha^{-1}. In the research, morphometric traits of fruit (weight, width and height), fruit firmness and soluble solids content were studied. Mentioned parameters were determined during the harvest of the examined cultivar and after 120 days of storage in a cooler at normal atmospheric pressure. In both years of investigation, two measuring were performed each (treated and non-treated (control variant), of which in the first year on October 9th and February 8th, and in the second year, on September 30th and January 30th.

Examinations of weight (g), height and width (mm), firmness of fruit (Pa; kg cm^{-2}), as well as the soluble solids content in the fruit (°Brix) were done by morphometric methods on a sample of 80 fruits (in four replications per 20 fruits). Fruit weight (g) is determined by measuring on a precision balance (Adventurer Pro AV812M, Switzerland), while length and width of fruit (mm) were determined by a digital caliper (Carl Roth, Germany). Firmness of the fruit is determined by a digital penetrometer (Model FHT-803, Italy), and the obtained values expressed in Pa, as well as by a penetrometer Bertuzzi FT-327 (Facchini, Alfonsine, Italy) with values expressed in kg cm^{-2}. The soluble solids content of the fruit of apple is determined by a binocular refractometer (Carl Zeiss, Jena) at room temperature (20 °C), and the values are expressed in °Brix.

The results are presented as mean ± standard error of mean (SE). Differences between mean values were compared by LSD test in three-way analysis of variance (ANOVA) using MSTAT-C statistical computer package (Michigan State University, East Lansing, MI, USA). Differences with p values of ≤ 0.05 were considered insignificant.

Results and Discussion

Research results on the impact of year, genotype and time of quality analysis on morphometric traits and soluble solids content of 'Granny Smith' apple in dependence of the application of foliar application 'Stopit' are shown in Table 1.

By analysis of variance all mentioned fruit characteristics are determined to be statistically significantly impacted by year and interaction of variability factors. Impact of genotype was not statistically significant in terms of fruit width, whereas the impact of quality analysis time is statistically insignificant for the soluble solids content.

Table 1. Impact of year, genotype and quality analysis time on morphometric traits and the soluble solids content of 'Granny Smith' apple in dependence of the 'Stopit' application

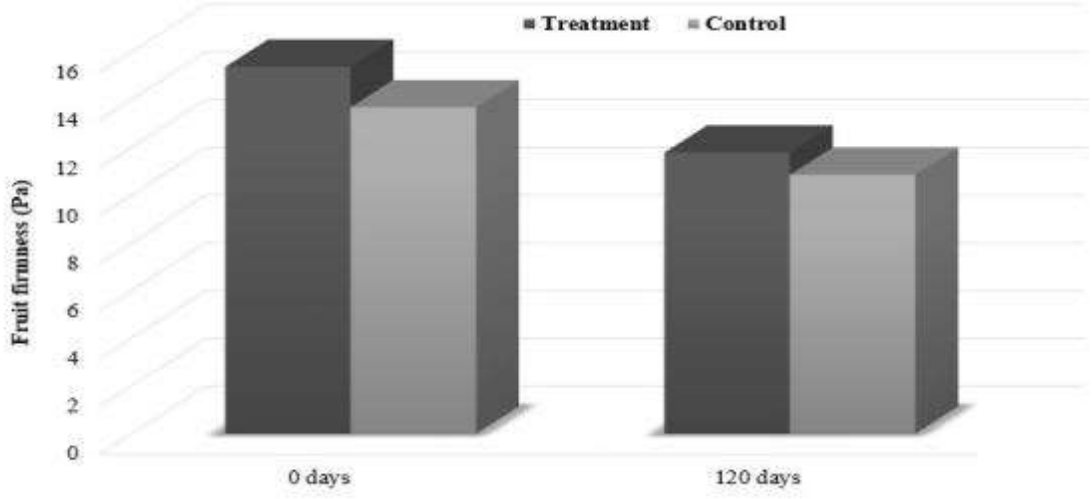
Treatment			**Fruit weight** (g)	**Fruit height** (mm)	**Fruit width** (mm)	**Soluble solids content** (°Brix)
Year (A)						
2018			190.65±2.71 a	69.23±0.50 a	78.64±0.40 a	11.58±0.14 b
2019			167.57±2.80 b	62.75±0.57 b	73.71±0.61 b	13.45±0.18 a
Genotype (B)						
Granny Smith			173.33±3.70 b	64.73±0.91 b	75.79±0.77 a	12.27±0.26 b
Control			184.89±3.85 a	67.25±0.97 a	76.57±0.85 a	12.76±0.31 a
Fruit quality analysis (C)						
0 days			183.85±3.87 a	66.97±0.87 a	77.62±0.56 a	12.51±0.31 a
120 days			174.37±3.87 b	65.01±1.04 b	74.74±0.87 b	12.53±0.26 a
Year × Genotype × Fruit quality analysis (A × B × C)						
2018	Granny Smith	0 days	188.85±3.43 bc	67.74±0.63 bc	78.92±0.53 ab	11.64±0.19 e
	Control	0 days	202.56±2.00 a	71.72±0.63 a	80.03±0.26 a	11.36±0.99 ef
	Granny Smith	120 days	179.72±6.34 bcd	67.99±0.86 b	77.22±0.35 bc	11.13±0.30 f
	Control	120 days	191.48±1.48 ab	69.49±0.40 b	78.41±1.09 ab	12.20±0.33 d
2019	Granny Smith	0 days	167.47±4.14 de	63.10±0.47 d	75.71±0.86 c	12.62±0.80 cd
	Kontrola	0 days	176.53±5.88 cd	65.34±0.72 c	75.82±0.62 c	14.41±0.17 a
	Granny Smith	120 days	157.28±3.60 e	60.11±0.68 e	71.30±0.45 d	13.70±0.13 b
	Control	120 days	168.99±5.48 de	62.47±0.80 d	72.02±0.72 d	13.08±0.14 c
ANOVA						
A			*	*	*	*
B			*	*	ns	*
C			*	*	*	ns
A × B × C			*	*	*	*

Values within each column followed by the same small letter are not significantly different at p≤0.05 by LSD test; ns - non-significant differences.

Average values of the fruit weight varied from 157.28 to 202.56 g, values of height and width ranged from 60.11 to 71.72 mm, that is, from 71.30 to 80.03 mm while the soluble solids content of the fruit varied from 11.13 to 14.41 °Brix. Observed by years, the greatest weight, height and width of the fruit were determined in the first year (190.65 g; 69.23 mm; 78.64 mm, in order), while the greatest soluble solids content were recorded in the second year of study (13.45 °Brix). On the other side, as regards genotype, values of the studied parameters were higher in control variant of the studied cultivar except in terms of the fruit width where no statistically significant differences were found. Considering the time of the fruit quality analysis, values of the studied parameters were greater at the time of harvest than after 120 days of storage, except for the soluble solids content where no statistically significant differences were found. As regards the determined statistical significance of all variability factors interaction, impact of interaction between individual factors cannot be interpreted independently. In interaction effect year/genotype/time of fruit quality analysis, average values of weight, height and width of the fruit varied significantly, with the highest values of tested parameters determined in the first year in control variant at harvest time and the lowest values in the second year of study after the analysis of fruits stored 120 days in a cooler. Weight of the apple fruit (*Malus* × *domestica* Borkh.) varies from 70 to 500 g, 98% of which is edible (Mišić, 1994). According to Mišić (2004), fruits of the apple cultivar 'Granny Smith' are classified into the group of middle-large to large (120–200 g). Asgharzade et al. (2012) reported that application of calcium chloride during vegetation affects the increase of apple fruit weight. Efficacy of the products containing calcium is reflected in a decrease of weight loss during the storage of fruits in a cooler (Mahajan and Dhatt, 2004). In this regard, Shirzadeh et al. (2011) emphasize that storing of treated fruits in a cooler leads to the loss of the fruit weight but that the losses are somewhat lower, compared to non-treated fruits, which has been confirmed in our research. Also, according to Gvozdenović (1998), fruits of the attractive apple cultivars are (supposed) to have dimensions of 65 to 75 mm, which is in accordance with the obtained results in this work. Morphometric traits of apple fruits are genetically conditioned, although their varying to a considerable extent may be caused by environmental factors (Krgović, 1990, Krpina et al., 2004). On the other hand, Amiri et al. (2008) point out that foliar application of some fertilizers can be more efficient regarding the impact on morphometric traits of the fruit and in comparison with standard fertilization method.

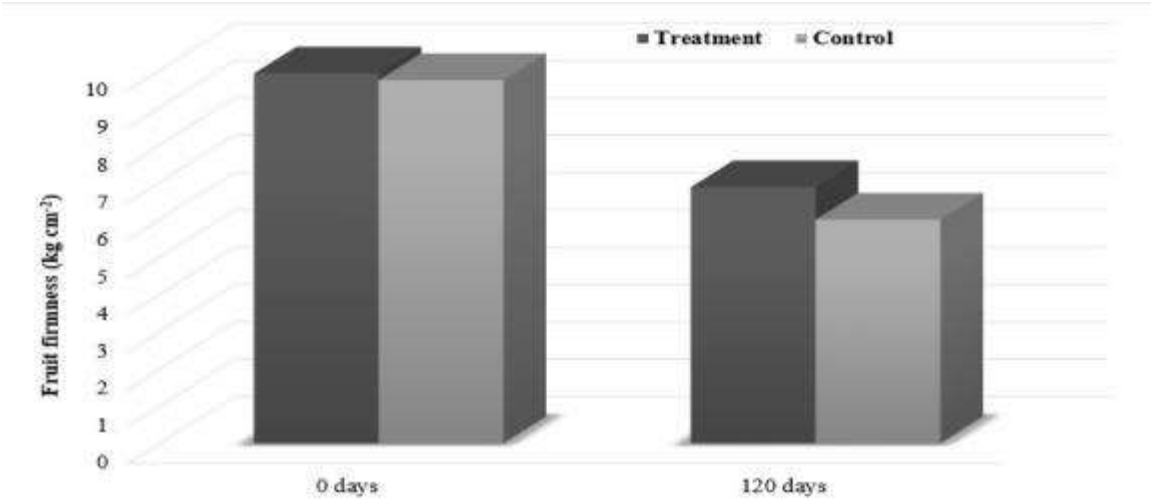
In interaction effect of variability factor, the highest average value of soluble solids content was recorded in 2019 in control variant at the time of fruit harvest while the lowest value of the respective parameter was recorded in 2018 in the variant of product application after the analysis of fruits stored for 120 days. Soluble solids content is one of the key parameters determining quality, and thereby the consumer acceptance. It increases with fruit ripening and is a basic indicator of quality and maturity. The content of soluble solids higher than 11,0% is considered the threshold of consumer acceptability for an apple cultivar and if the values of the respective parameter is greater than 13,5% , consumer acceptance is better. Treatments with calcium are assumed to decrease the soluble solids content in apple fruits during the storage period due to the breathing slowdown caused by cooler temperatures and thus, a slower conversion of starch into sugars (Rohani et al., 1997) However, Netravati et al. (2018), reported that after the mentioned treatments and apple fruit storage in a cooler, the content of soluble solids is increased accordingly. Research results of the effect year, genotype and time of quality analysis on the firmness of 'Granny Smith' apple fruit in dependence of the foliar application 'Stopit' are shown in Graphics 1 and 2. Application of 'Stopit', which in its chemical composition contains calcium in the form of calcium chloride, exhibited a positive effect on the firmness of the studied cultivar in both years of study. In the first year, the greatest fruit firmness was recorded at the harvest of the studied cultivar (15.39 Pa), and was

higher in relation to the control (13.69 Pa). Value of the tested parameter during the storage period of 120 days in a cooler decreased (11.79 Pa), although in that case, a higher value compared to the control variant at the same time of study was recorded (10.86 Pa).



Graphic 1. Impact of genotype and time of fruit quality analysis on the firmness of 'Granny Smith' apple fruit

In the second year of study, the greatest firmness of the fruit was recorded at harvest time (9.91 kg cm^{-2}). After 120 days of storage in a cooler, value of the respective parameter decreased (6.86 kg cm^{-2}). Recorded values in both study terms were higher in the variant with 'Stopit' application compared to the control variant (0 days - 9.73 kg cm^{-2}; 120 days - 5.99 kg cm^{-2}).



Graphic 2. Impact of genotype and time of fruit quality analysis on the firmness of 'Granny Smith' apple fruit

Fruit firmness is one of the crucial physical properties that is, an indicator of fruit maturity dictating the length of fruit storage as well as their timely distribution to the market. Apple producers are enabled to extend the time of apple distribution to the market by storing in coolers and slowing down their ripening i.e., maintaining the fruit firmness stability. The research results obtained in this work are in accordance with the quotes of Asgharzade et al.

(2012) that treating apples with calcium chloride prior to harvest significantly affects the increase of the flesh firmness. In addition, Benavides et al. (2002) and Casero et al. (2004) quote that their application decreases softening and keeps fruit firmness of apple fruits during storage. Positive effects of calcium chloride application on the increase of fruit firmness are most likely connected with the content of calcium chloride in the fractions of covalently linked pectins (Siddiqui and Bangerth, 1995). Furthermore, calcium affects the stabilization of cell membranes, thus preventing physiological disturbances ascribed to its lack (Saure, 2005).

Conclusions

Foliar application of 'Stopit' contributes to the improved quality and preservation of the optimal apple fruit quality. The two-year foliar application of the 'Stopit' in cv 'Granny Smith' had a positive effect on the most important indicators of fruit quality, i.e., specific pomological traits of apple. In this regard, producers may be given some guidelines in improving the cultivation technologies through application of 'Stopit', with the aim of more qualitative storage of apple fruits.

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THE INFLUENCE OF NITROGEN ON THE YIELD AND QUALITY OF CARROTS

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Abstract

Carrots belong to nitrophilic plants. It deposits nitrogen in the roots and leaves. In carrot production technology, the proper use of mineral fertilizer is very important. Improper nitrogen fertilization causes nitrate to accumulate at the root of the carrot. The aim of the study was to show how increasing doses of nitrogen affect the quality and yield of carrots. Doses of nitrogen were analyzed (ø, 80 kg N/ha, 160 kg N/ha, 240 kg N/ha). Varieties were tested (*Almaro F1*, *Chantenay*, *Flakkee*). The results of the experiment show that increasing doses of nitrogen have a negative effect on the quality of carrots. The highest accumulated nitrogen (0,059%) was in the variant 240 kg N/ha. The least accumulated nitrogen was in the control variant (0,050%). the potassium content, depending on the fertilizer, was moving in the direction of falling. A nitrogen dose of 80kg/ha did not significantly affect yield.

Keywords: *carrots, nitrogen fertilization, yield, quality*

Introduction

Carrot have great importance in human nutrition. It is rich in vitamins and minerals. Carrot can be used fresh, cooked or processed. It is among the ten most important vegetable crops. It is especially important in the nutrition of children. High levels of provitamin A in carrots are important for treating skin diseases and poor eyesight. *(Đurovka, 2008).* It belongs to the nitrophilic species which means that it collects large amounts of nitrate in the root and leaf. Carrot yield and nitrate content depend on correct nitrogen fertilization. The quality of carrots depends on the correct dosage of nitrogen during the growing season. Nitrate accumulation at the roots of carrots will not be large if the nitrogen fertilization dose is not exceeded 150 kg ha^{-1} *(Šunić, 2014)*. The aim of the study was to show how increasing doses of nitrogen affect the quality and yield of carrots.

Material and methods

In an experiment during 2016, the influence of increasing doses of nitrogen on the chemical composition of carrots was analyzed. The experiment was set up by random block system in four repetitions. Doses of nitrogen were analyzed (ø, 80 kg N/ha, 160 kg N/ha, 240 kg N/ha). Varieties were tested (*Almaro F1*, *Chantenay*, *Flakkee*). The size of the experimental plot was $2m^2$ (1mx2m). The sowing was in the first decade of March in 2016. The distance between the plants was 5cm, and the distance between the rows was 20cm. A total of 10 000 plants per/$100m^2$. The basic characteristic of the land on which the experiment was set up were: 4,91 pH (in KCl); <1% $CaCO_3$;4,54% humus; 0,76 mg $100g^{-1}$ Al- P_2O_5 i 37,40 mg $100g^{-1}$ Al-K_2O. In the autumn, calx (15,8 kg/100 m^2) was introduced into the soil to reduce acidity.The analysis of plant material was done in the laboratory of the Faculty of Agriculture in Banja

Luka. Nitrogen was analyzed, according to Kjeldahl, phosphorus on spectrophotometer, potassium - wet burning of plant material with a mixture of acids (HNO_3 + $HClO_4$ + H_2SO_4), concentration reading by the AAS method, model Unicam SP9. The obtained results were processed by the variance analysis method for a two-factor experiment (ANOVA). The significance of differences between individual environments was tested by LSD test.

Results and Discussion

The analysis showed that the highest yield was on the fourth fertilizer variant (817,50 kg/100m^2), then on the second (810,83 kg/100m^2) and third variant (717,50 kg/100m^2) (table1). No difference in yield was found between the second and third fertilizer variants. Nitrogen levels greater than 100 kg/ha did not affect the yield increase *(Marković i sar., 1992)*. Similar results were published by Wiebe, (1987). This author claims that the highest carrot yield was achieved by applying nitrogen fertilizer of 80-140 kg/ha. Similar results are reported by *Galeev et al.(1980)*; *Ilin et al. (2004); Krasnić (1998);R.Scott Veitch et al.(2014).*

Table 1. Influence of fertilization and varieties on carrot yield (kg/100m^2)

Nitrogen fertilization	Variety			Average for nitrogen fertilization
	Almaro F_1	Chantenay	Flakkee	
ø	545	271,87	800	538,95
N_{80} (80kg N/ha)	530	915	987,5	810,83
N_{160}(160kg N/ha)	637,5	825	690	717,5
N_{240} (240kg N/ha)	727,5	825	900	817,5
Average for variety	610	709,21	844,37	721,19
LSD	A	B	AxB	
0.05	141,22	122,31	244,63	
0.01	190,38	164,89	329,78	

The chemical composition of carrot roots is a varietal characteristic. However, the chemical composition is influenced by various factors of which fertilization has the greatest influence *(Kaack et al., 2001; Singh et al., 2012; Smoleń et al., 2012)*. The maximum nitrogen content (0.059%) was determined in the fourth fertilizer variant 240 kg N / ha, while the minimum content was recorded in the control variant (0.050%). The differences in nitrogen content between these variants were statistically significant.

Table 2. Influence of fertilization and varieties on % of nitrogen in the root of carrots

Nitrogen fertilization	Variety			Average for nitrogen fertilization
	Almaro F_1	Chantenay	Flakkee	
ø	0,060	0,047	0,045	0,050
N_{80} (80kg N/ha)	0,062	0,042	0,052	0,052
N_{160}(160kg N/ha)	0,062	0,040	0,060	0,054
N_{240} (240kg N/ha)	0,067	0,047	0,062	0,059
Average for variety	0,063	0,044	0,055	0,054
LSD	A	B	AxB	
0.05	0,004	0,003	0,007	
0.01	0,005	0,004	0,009	

Phosphorus content values range of 0.055% to 0.275% (tab.3). The highest potassium content was recorded on the control variant (0,558%), while the lowest potassium (0,385%) content was recorded on the fourth fertilizer variant. The differences in potassium content between the control variant and other fertilizer variants were statistically highly significant.

Table 3. Influence of fertilization and varieties on % of phosphorus in the root of carrots

Nitrogen fertilization	Variety			Average for nitrogen fertilization
	Almaro F_1	Chantenay	Flakkee	
ø	0,055	0,037	0,050	0,047
N_{80} (80kg N/ha)	0,045	0,027	0,027	0,033
N_{160}(160kg N/ha)	0,245	0,187	0,190	0,207
N_{240} (240kg N/ha)	0,275	0,195	0,257	0,242
Average for variety	0,155	0,111	0,131	0,132
LSD	A	B	AxB	
0.05	0,021	0,059	0,037	
0.01	0,029	0,079	0,050	

Potassium is of particular importance in plant life, directly or indirectly affects a number of physiological-biochemical processes in the plant *(Kastori at al., 2013)*. More specifically, nitrogen is an exception among nutrients. Plants adopt nitrogen in the form of cations (NH_4^+), anions (NO_3^-) or in organic form. This property of nitrogen significantly affects the mineral nutrition of the plant and the uptake of potassium, namely, potassium is adopted only in the form of cations. According to research by *Kastori et al., (2013)* cation NH4 + decreases cation uptake, and nitrate anion NO3- decreases anion uptake. In their research *(Nieves-Cordones et al., 2007)*, it concludes that the increase of nitrogen fertilizer also increases the amount of NH4 + ions in the soil, and decreases the uptake of potassium cations. Their research is in line with ours (tab.4).

Table 4. Influence of fertilization and varieties on % of potassium in the root of carrots

Nitrogen fertilization	Variety			Average for nitrogen fertilization
	Almaro F_1	Chantenay	Flakkee	
ø	0,590	0,527	0,557	0,558
N_{80} (80kg N/ha)	0,460	0,402	0,375	0,412
N_{160}(160kg N/ha)	0,400	0,365	0,525	0,430
N_{240} (240kg N/ha)	0,390	0,420	0,345	0,385
Average for variety	0,460	0,428	0,450	0,446
LSD	A	B	AxB	
0.05	0,037	0,032	0,064	
0.01	0,050	0,043	0,086	

Conclusions

The best quality of carrots was obtained by applying nitrogen fertilizer in quantities of 80 kg/ha. Fertilizing with larger amounts of nitrogen adversely affected the potassium content of the carrot root. With the increase of nitrogen units, the amount of potassium decreased. The highest value of potassium was recorded on the control variant. The lowest value of this mineral was recorded on the variant where the highest dose of nitrogen units was applied. The

highest nitrogen accumulation was recorded in the variant with the highest dose of nitrogen units. Nitrogen levels above 80 kg/ha did not significantly increase yield.

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EXPLORATION OF VITICULTURAL TASKS TO BE PERFORMED BY AN AUTONOMOUS ROBOT: POSSIBILITIES AND LIMITATIONS

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Abstract

In the past few decades, new technologies have entered the agricultural fields providing means of effectively managing vineyards, improving production quality and minimizing resources, while reducing production costs and achieving sustainability. In this context, robotics has been developed to support agriculture and already agricultural robots, namely *Agrobots*, are available in the market. Among several projects taking place worldwide, a prototype autonomous agrobot is currently being developed aiming to be used for vineyard management and harvesting grapes in vertical training systems. The agrobot of our interest combines commercial hardware and machine vision, contributing towards a reliable information acquisition system that includes sensor-fusion algorithms and data analysis. Seasonal cultivation practices applied to the vineyards include shoot removal, leaf removal and cluster thinning. Automation of these viticultural tasks, including harvesting, by means of agrobots is an increasing trend, characterized by efficiency and improvement of production quality. In this paper, the possibilities of implementing an autonomous agricultural robot for supporting vineyard management are discussed, focusing on summer pruning, i.e. leaf removal, cluster thinning and harvesting. In addition, limitations are presented, taking under consideration the conditions in Northern Greek vineyards. This work is based on interviews of expert agronomists and enologists of local wineries, and on the characteristics of 9 vineyards located in Northern Greece, based on vineyard management/ harvest reports and vineyard mapping. This study is part of the ongoing work of the national co-funded project POGHAR (Personalized Optimal Grape Harvest by Autonomous Robot).

Keywords: *leaf removal, harvesting, robot, vineyard, viticultural practices.*

Introduction

It is generally accepted that the quality of grapes is determined by three categories of factors, i.e. the characteristics of the variety, the elements of the natural environment and the viticultural practices applied. The density of the vegetation affects the shading and consequently the microclimate in the different parts of the vegetation. Canopy manipulation and summer pruning (e.g. hedging, basal leaf removal, lateral shoot removal, cluster thinning) are crucial and have an impact on improving product quality, grape and wood ripening (maximum photosynthetic activity, favorable microclimate), bud fertility (increased brightness) and preventing or limiting the development of some diseases. In addition, interventions in the vegetation are necessary in the cases of mechanization, in order to reduce production costs (Reynolds, 2010; Verdenal et al., 2017; Yu et al., 2016).

Agricultural robots are increasingly used to support human labour in the fields, providing automated solutions to tedious and elaborate tasks. Agrobots are assigned to carry out harvesting and other cultivation practices, due to their advanced modularity, robustness and accuracy with the dexterity of a skillful human harvester (Matese and Di Gennaro, 2015). The development of an agrobot requires machine vision algorithms and calibration models that can be provided by precision viticulture techniques, integrated into a robotic system capable to navigate into the vineyard autonomously and perform a variety of tasks, including harvesting (Mavridou *et al*. 2019). Recent studies have demonstrated the capability of machine vision techniques for object detection and localization; fast and accurate algorithms have been developed to detect groups of grapes, their shape and volume, the size of the clusters and even the ideal cutting point for their harvest (Badeka *et al.*, 2019; Kalampokas *et al.*, 2020). The practical development of robotics in precision viticulture is still in its infancy, however, many projects are already underway, and some are already on the market. Several commercial robots have been proposed in recent years for vineyard management; for data collection (such as multispectral pointers or images) or for certain functions such as pruning. Harvesting capabilities are also developed (Matese and Di Gennaro, 2015). Although purchase and operating costs of agrobots may be high in the early years, improving them will certainly be a milestone for the precision of automated solutions, since precision leads to undamaged grapes and thus, to enhancement of the quality of the grapes. Yet, there are significant problems that need to be resolved. The objective of the present paper is to investigate the application of a developing agrobot in vineyard management,particularly in leaf removal, cluster thinning and harvesting. This work focuses on the specific requirements of these practices, based on interviews of expert agronomists and enologists of local wineries, and on the characteristics of vineyards located in Northern Greece in terms of vineyard management/ harvest reports and vineyard mapping.

Material and methods

Data have been collected through a) interviews with 9 agronomists and enologists of three medium size wineries of Northern Greece (regions of Drama and Kavala), b) reports of viticultural tasks implemented 5 previous years in each vineyard (3 vineyards per winery), c) mapping of 9 vineyards (3 vineyards per winery), cultivated with different white or red varieties. The face-to-face survey was conducted during 2019. A specialized autonomous agrobot will be developed by HUMAIN-Lab of the International Hellenic University able to automate viticultural tasks, such as harvest, cluster thinning and leaf removal. This robot aims at developing on-board intelligent decision making on-the-spot based on commercial hardware, machine vision and innovative computational intelligence algorithms.

Results and discussion

According to the characteristics of agricultural robot, the following viticultural tasks would be appropriate to be performed by it.

Leaf removal: Leaf removal consists of removing a number of leaves from the base of the shoots in order to better expose the grapes to the sun. It mainly aims at modifying the microclimate of foliage and grapes and restore a balanced relationship between shoots and reproductive organs of the vine to achieve a proper quality and quantity of production. It results to the improvement of the ripening of the grapes, the improvement of the ventilation and the efficiency of the sprays (powdery mildew, Botrytis, grape berry moth), as well as the assistance of the harvest. It is a very effective practice, as long as it is used rationally and depending on the place and the variety (eg. removal of less leaves for white early varieties to

avoid loss of aromatic potential, more in later red varieties for color increase and restriction of plant aromas etc.) and the removal of grapes (cluster thinning) usually at the stage of ripening (Poni et al. 2016; Sivilotti et al. 2014).
Good defoliation practice, with regard to time and intensity, depends upon: a. the area, as the intensity is lower in hot areas, higher in mild climate areas and necessary for late varieties and / or in cold areas, b. the season, as it is best to be applied at the beginning of the period for better development of cortex strength, while in hot areas is performed later, in the middle of the season and closer to ripening for late-ripening varieties, c. the variety and the desired wine product, as it should be less intensive for white and early-ripening varieties, mild for red grape varieties and more intensive for late (mainly red) varieties, d. the orientation of the lines, as it affects the intense of sunlight received by the plants and therefore, should be performed from the north side, when the lines are oriented in the E-W direction, and from the east side, when the lines are oriented N-S. (Constantinou et al. 2015).
Based on the above, the removal of leaves by the autonomous robot can be realized in the cluster area, i.e. from the base of the shoot up to 5-7 knots. The work could be described as a percentage, based on vegetation indices (Normalized Difference Vegetation Index - NDVI) in the cluster zone detected by the multispectral camera, or as a desired foliage density. In addition, uniform - in space - leaf cutting according to identification methods (e.g. based on convolutional neural networks– CNNs algorithms) may be proposed.



Figure 1: Application of leaf removal in red grape variety

***Cluster Thinning*:** Cluster thinning concerns the exclusion of unripe grapes, parts of them or individual berries, in order to improve the relationship between foliage and yield. The choice of the specific part to be removed depends on the intended purpose and the properties of the variety (eg. size and shape of grapes). Cluster thinning is necessary not only on table varieties, but also on wine varieties intended for the production of quality wines. It is actually legally imposed on vineyards used for producing Protected Denomination of Origin (PDO) wines, especially nowadays that viticultural practices result in the production of a larger number of clusters per vine (Filippetti et al. 2007; Keller et al. 2005; Sun et al. 2012).
The removal of part of the grape bunches is applied mostly in red wine varieties at veraison stage and after the vegetation growth has been suspended. In this way, better utilization of photosynthetic function is sought for the benefit of production. As far as the vine is concerned, the differentiation of the buds is favored, a balance between vegetation and production is achieved, while the vine carbohydrate status increase, which affect the smooth and satisfactory fruiting of the following years (Pellegrino et al. 2014). Based on the above, cluster thinning by the autonomous robot can be performed based on the multispectral images as a percentage subtraction of the total number of grape bunches on the vine. The grapes could be selected to be removed by calculating the average size of the bunch and removing those on the outer edge (e.g. very small).



Figure 2: Application of cluster thinning in red grape variety

Determination of ripeness and selection of suitable grapes for harvest: Grape maturation includes the technological (desired concentration of sugars and acids in the must), the phenolic (concentration of mainly anthocyanins and tannins in the berry skins and seeds) and the aromatic maturity (volatile compounds that form the "varietal character" and designate the intensity and complexity of wines). It is understood that maturity is not a fixed stage, on the contrary it is a conventional concept that varies depending on the type of wine that will be produced. In order to identify the appropriate stage for harvest, it is necessary to monitor the ripening starting at the stage of veraison, since it is particularly important for the better organization of the available resources on the part of the winery. The key to a good assessment of the ripening level is to collect a sample as representative as possible of all the grapes in the vineyard (average ripening state of the whole vineyard). In addition to the already known analyses performed during the estimation of the maturation process, in the context of the automation of the aforementioned process, measurements of temperature, pH value and soluble content could be made, which in combination with images taken from a constant distance / height, would be useful and reliable tools in order to identify the appropriate date for harvesting. Rabot (2017) also used chemical analysis and image analysis to evaluate the phenolic maturity of seeds without biochemical analysis, in order to determine the optimal harvest date for the most favorable tannin extraction conditions and, consequently, the best organoleptic characteristics. Fernandes et al. (2015) applied a methodology with hyperspectral data and neural networks that allowed the simultaneous determination of pH value, sugar content and anthocyanins concentration in grape berries of red cv. Touriga Franca. Correlation of images to phenolic characteristics, presupposes acquisition of multiple images during ripeness, from veraison to harvest time, and corresponding samplings and chemical analysis on the samples, making the in-field task of image acquisition particularly detailed and demanding. This pre-processing step is an ongoing task in the context of POGHAR project.

Limitations

This section records the concerns of agronomists for the automation of viticultural processes by agricultural robots.

1. Leaf removal: Due to the practice to remove inner leaves of the foliage in white cultivars and retain the outer, in order to shade and protect the grapes from sunlight it would be challenging to remove the inner leaves and retain the external. On opposite, red cultivars are treated differently, as external foliage is removed, however not entirely.
2. Cluster thinning: The agrobot should be able to identify which bunches should be cut, taking into account, firstly the hygiene condition of the grapes, secondly the right size and third the degree of ripeness.

3. Sampling: If sampling is performed, the agrobot would probably face difficulties in removing the berries from the inner (back) of the grape bunch.
4. Harvest: Considerations at harvesting include the ability of agrobot to cut the bunch stem without injuring the berries and the ability to select among red grapes these appropriate grapes for rosé vinification.
Some additional concerns and problems that the robot may encounter during performing its tasks are presented in Table 1.

Table 1. Problematic areas in robot use

Concerns/ possible problems	**Ability the robot should have:**
Several vineyards are established on slopes	Move on slopes
Ground may be uneven and, in some vineyards, may contain a large percentage of stones	Move on uneven ground
Heavy rainfall	Move on heavy ground
Some varieties (eg. cv. Sauvignon blanc) have very small bunch stem	Cut the bunch stem without injuring the grapes
Transportation of grapes during harvest, collection of crates	Transport the crates during harvest
Speed/performance of robot/ duration of autonomy	Satisfactory cutting/harvesting speed and autonomy

*Source: Author's elaboration based on interviews with viticulturists

Conclusions

Agricultural robots are being developed for the dynamic conditions of agricultural environments, such as vineyards. This work addresses the possible application of the developing agrobot in vineyard management and particularly in leaf removal, cluster thinning and harvesting. In each case, the appropriate software would allow the agrobot to select a) the leaves that should be removed without having a negative effect in grape quality, b) unsuitable grapes (infected by disease or small) for cluster thinning and c) grapes ripe enough for harvesting during the homogeneous harvest. Limitations that should be addressed with the development of software / tracking algorithm are also noted.

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APPLICATION OF V AND D CRYO-PLATE METHODS FOR THE CRYOPRESERVATION OF CHERRY ROOTSTOCK GISELA 5

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Abstract

The possibility of improving cryopreservation success using aluminium cryo-plates was investigated in cherry rootstock Gisela 5. The shoot tips were dissected from *in vitro*-grown shoots and precultured for one day at 25 C in the dark, on Murashige and Skoog (MS) medium containing 0.3 M sucrose. The explants were placed on aluminium cryo-plates with 12 wells and embedded in 2% alginate gel. Osmoprotection was performed by immersing the cryo-plates in loading solution (1.9 M glycerol + 0.5 M sucrose) for 30 min at room temperature. In the V cryo-plate protocol, dehydration was performed at room temperature using two types of plant vitrification solutions: modified PVS2 (37.5% glycerol, 15% dimethyl sulfoxide, 15% ethylene glycol and 22.5% sucrose) for 40 min and PVS3 (50% glycerol and 50% sucrose) for 60 min. In the D cryo-plate protocol, explants were desiccated for 2, 2.5 or 3 h in closed glass containers over silica gel. In both protocols, cryo-plates with explants were transferred in uncapped cryotubes and directly plunged into liquid nitrogen. Rewarming was done by direct immersion of cryo-plates in liquid MS medium containing 0.8 M sucrose (30 min at room temperature). In the V cryo-plate procedure regrowth of cryopreserved shoot tips dehydrated with PVS2 was 95.9%, while for those dehydrated with PVS3 was 85%. As for the D cryo-plate procedure, regrowth ranged between 45.8% and 66.7%. After regrowth, shoots were successfully multiplied and rooted. The results obtained clearly indicate that both cryopreservation procedures using aluminium cryo-plates can facilitate efficient cryostorage of Gisela 5 rootstock.

Keywords: *In vitro conservation, Aluminium cryo-plates, Vitrification, Air dehydration.*

Introduction

Tissue culture techniques are not only being commonly applied for collection, rapid multiplication and exchange of plant material, but also for short- and long-term conservation of vegetatively propagated plant species, including different fruit crops. Application of *in vitro* techniques for conservation of plant genetic resources started in the early 1980s with development of slow growth techniques (Engelmann, 2011) mostly intended for medium-term storage due to the likelihood of genetic instability and possible losses caused by contamination in the process of repeated subculturing during long-term period (González-Benito *et al*., 2004).

Cryopreservation is an effective approach for long-term germplasm storage at ultra low temperatures in liquid nitrogen (LN, −196 °C), thus ensuring no metabolic processes to occur (Reed, 2017). In this way, plant material can be conserved in principle indefinitely, with no need for subculturing and with reduced threat of somaclonal variation. In the last three decades a number of different cryopreservation protocols, from the original slow cooling approach to easier and more reproducible vitrification-based cryopreservation techniques such as encapsulation-dehydration, vitrification, encapsulation-vitrification and droplet vitrification have been developed and utilized for germplasm storage (Agrawal *et al*., 2019). Vitrification-based cryopreservation techniques eliminate the need for expensive, programmable freezing equipment (controlled-rate freezers) and permit tissues to be cryopreserved by direct transfer to LN. Implementation of these

techniques has extended the applicability of cryopreservation to a broad range of plant species including different *Prunus* species (Benelli *et al*., 2013). Vitrification and droplet-vitrification techniques were also successfully applied for cryopreservation of cherry rootstock Gisela 5 (Ružić et al., 2013; Ružić *et al*., 2014). However, these methods require skilful manipulation to avoid damage and subsequent loss of shoot tips as well as insufficient or excessive dehydration during procedure (Yamamoto *et al*., 2011).

Recently, two efficient and simple cryopreservation methods using aluminium cryo-plates (V cryo-plate and D cryo-plate) have been developed. V cryo-plate method (Yamamoto *et al*., 2011) is based on dehydration of explants with Plant vitrification solution 2 (PVS2; Sakai *et al*.,1990) while D cryo-plate method (Niino *et al*., 2013) is based on dehydration in the air current of the laminar flow cabinet or over silica gel (desiccation). Both protocols appear promising for cryopreservation of both herbaceous and woody plants (Niino *et al*., 2019) including different fruit tree species after appropriate modifications of the procedures. They were successfully applied to strawberry (Yamamoto *et al*., 2012a), mulberry (Yamamoto *et al*., 2012b), blueberry (Dhungana *et al*., 2017), as well as cherry plum and European plum (Vujović *et al*., 2015).

Although previous research on cryopreservation of cherry rootstock Gisela 5 showed that vitrification and droplet-vitrification techniques can be successfully applied for long-term storage of *in vitro* shoot tips of this genotype (Ružić *et al*., 2013; Ružić *et al*., 2014), the present study was intended to simplify cryopreservation procedure and to improve survival and regrowth success using V cryo-plate and D cryo-plate methods.

Material and Methods

Tissue-cultured shoots of cherry rootstock Gisela 5 (*Prunus cerasus* × *Prunus canescens*) were used for the experiments. Aseptic culture of this genotype was established according to the protocol previously described by Ružić *et al*. (2015) and shoots were multiplied on Murashige and Skoog (1962) medium (MS) containing 3.37 mg l^{-1} N6-benzyladenine (BA). Cultures were maintained in a growth chamber at 23 ± 1°C (16 h photoperiod, light intensity 54 µmol m^{-2} s^{-1}) and subcultured at 3-week intervals to obtain a sufficient number of plantlets for cryopreservation experiments. Excised axillary buds were precultured for 1 day at 23 ºC in the dark on solidified MS medium with 0.3 M sucrose.

Precultured explants were placed in wells on aluminium cryo-plates previously filled with 2% (w/v) sodium alginate in calcium-free MS basal medium with 0.4 M sucrose (about 4 µl). MS basal medium containing 0.1 M calcium chloride and 0.4 M sucrose was poured on the aluminium plates over shoot tips and left for 20 min to achieve complete polymerization of sodium alginate. Cryo-plates with explants were transferred to loading solution (LS) with 1.9 M glycerol and 0.5 M sucrose in liquid MS medium for 30 min at room temperature.

The V cryo-plate procedure was performed using PVS A3 (22.5% sucrose, 37.5% glycerol, 15% ethylene glycol and 15% dimethyl sulfoxide) (Kim *et al*. 2009) for 40 min, or PVS3 (50% glycerol and 50% sucrose) (Nishizawa *et al*. 1993) for 60 min. Dehydration with both types of VSs was done at room temperature while durations of treatments were selected as the most efficient ones in the previous droplet-vitrification experiments (Ružić *et al*., 2013).

In D cryo-plate procedure dehydration included desiccation of the explants attached to the cryo-plates in closed containers over 40 g of silica gel at 23°C for 2, 2.5 and 3 h.

After dehydration, in both protocols cryo-plates with explants were transferred to 2 ml uncapped cryotubes, directly immersed in LN and kept for 1 h. For rewarming, aluminium cryo-plates were immersed in an unloading solution (0.8 M sucrose) for 30 min at room temperature. Then, explants were transferred onto the regrowth medium (MS medium with 3.37 mg l^{-1} BA), cultivated in the dark for seven days, and then under standard conditions.

Each critical step in both procedures had proper control: pregrowth control – after pregrowth explants were directly transferred onto the regrowth medium; loading control – explants

exposed to loading solution but neither dehydrated nor cryopreserved; dehydration controls – following loading explants were dehydrated with VS or desiccated and directly unloaded without immersion in LN.

Survival was evaluated two weeks after samples retrieval from LN by counting the number of explants showing any signs of regeneration. Regrowth was defined as further development of apices into viable shoots up to the sixth week. Each experimental treatment was replicated three times with each replication containing 10–12 shoot tips. Statistical analysis was performed using one-way ANOVA and then Duncan's Multiple Range Test ($P < 0.05$) to compare the means. Data presented in the form of percentage were subjected to arcsine transformation before analysis of variance.

Shoots originating from different treatments were separately transferred onto the MS multiplication medium. Multiplication index and length of axial and lateral shoots were monitored in the third subculture after regrowth. In the following subculture the shoots were rooted on the medium with ½ MS mineral salts, organic complex unchanged, 1 mg l^{-1} 1-naphthaleneacetic acid and 0.1 mg l^{-1} gibberellic acid. The following parameters were monitored: percentage of rooting, number and length of roots, and length of rooted shoots.

Results and Discussion

In the present study, V cryo-plate and D-cryo-plate methods were successfully applied to *in vitro* shoot tips of Gisela 5 cherry rootstock. During cryopreservation process we monitored both survival (presence of green tissue) and regrowth of explants (shoot growth and development) after every critical step in both protocols (Table 1). Namely, pregrowth, osmoprotection, exposure to highly concentrated VSs (V cryo-plate) or air dehydration (D cryo-plate) are of vital importance for successful cryopreservation, but each of these steps also affects viability of non-cryopreserved explants.

As regards survival, no significant differences were observed among pregrowth, loading, dehydration controls and cryopreserved explants (Table 1). Survival rates were very high and ranged between 95.9% and 100%. However, loading treatment significantly reduced regrowth capacity of explants (90%) in comparison with pregrowth control (100%; Table 1). In the V cryo-plate protocol dehydration treatments (PVS A3 and PVS3) did not further affect regrowth of control explants (90%). After cryostorage, significantly higher regrowth rate was obtained in explants dehydrated with PVS A3 solution (95.9%) in comparison with those dehydrated with PVS3 (85%). Plantlets, regenerated after LN exposure, were vigorous and displayed normal morphology (Figure 1a–b). Regrowth rates of dehydration controls (both PVS A3 and PVS3) and cryopreserved shoot tips achieved in our experiment are more than twice higher than those obtained by Ružić *et al.* (2013) in droplet-vitrification experiments conducted under the same dehydration conditions. Similarly, Vujović *et al.* (2015) reported significant improvement in the regrowth of cryopreserved explants of cherry plum using V cryo-plate method. Yamamoto *et al.* (2011) assumed that calcium alginate gel used to attach explants to aluminium plates mitigates the cytotoxicity of PVSs. In addition, V cryo-plate method enables more precise time control of dehydration and reduces possibility of injuring shoot tips during manipulations.

In contast to results obtained with V cryo-plate method, considerable lower regrowth rates in control (50–83.3%) and frozen explants (45.8–66.7%) were achieved using D cryo-plate method (Table 1). The highest regeneration ability of both types of explants was obtained after 2.5 h of desiccation. In all experimental treatments shoots regenerated from cryopreserved explants were vigorous, but exhibited symptoms of hyperhydricity (Figure 1 c–e). According to Niino *et al.* (2014) the D cryo-plate method may be used with larger explants, that enables more uniform physical dehydration. Further, optimisation of hormonal composition of recovery

medium and adding antioxidants to the cryoprotectant and/or to recovery medium may improve regrowth after LN exposure (Uchendu *et al.*, 2010).

Table 1. Survival and regrowth of pregrowth, loading and dehydration controls (-LN) and cryopreserved explants (+LN) of Gisela 5 rootstock using V and D cryoplate methods

Treatment/duration	Survival (%)		Regrowth (%)	
	-LN	+LN	-LN	+LN
Pregrowth control	100.0*	-	100.0 a	-
Loading control	100.0	-	90.0 bc	-
PVS A3/40 min room t°	100.0	100.0	90.0 bc	95.9 ab
PVS3/60 min room t°	100.0	100.0	90.0 bc	85.0 c
2 h desiccation	100.0	95.9	58.3 ef	45.8 f
2.5 h desiccation	100.0	95.9	83.3 c	66.7 de
3 h desiccation	100.0	100.0	50.0 ef	66.7 de
Significance	ns		P < 0.05	

Mean values for regrowth followed by the same letter are not significantly different according to Duncan's Multiple Range Test; ns – non significant.

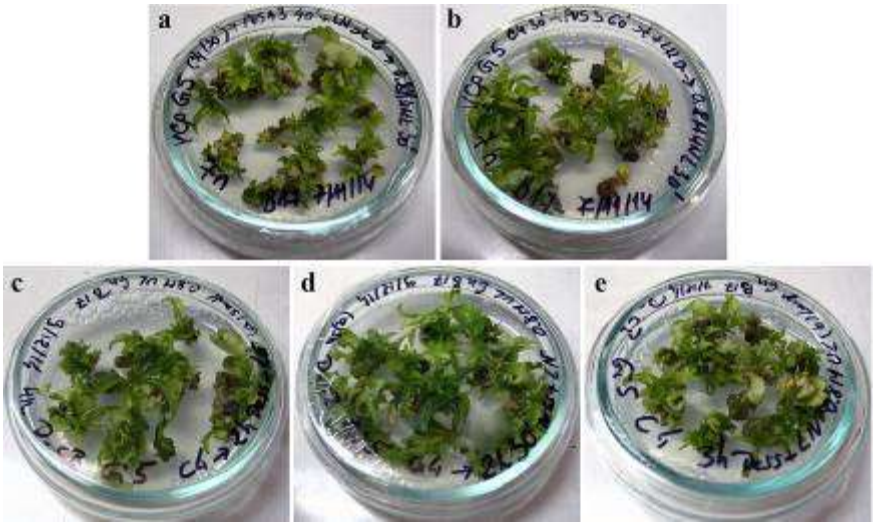


Figure 1. Regrowth of cryopreserved shoot tips of Gisela 5 rootstock: explants dehydrated with PVS A3 for 40 min (a) and with PVS3 for 60 min (b); explants desiccated for 2 h (c), 2,5 h (d) and 3 h (e)

With consideration given to factors of high survival, high regrowth, and simplicity of the protocols, both methods have been demonstrated as a highly effective for cryogenic storage of Gisela 5 rootstock. However, successful utilisation of any cryopreservation technique dependends not only on efficient regrowth after thawing, but also on efficient propagation and subsequent rooting of true-to-type regenerants. In our experiments, loading as well as all PVS and desiccation treatments significantly increased capacity for multiplication of regenerated shoots in comparison with those originated from pregrowth controls (Table 2). Cryopreservation slightly decreased multiplication index of regenerated plantlets compared with corresponding controls. The only exception was PVS A3 treatment where shoots originated from cryopreserved explants displayed the highest multiplication capacity (index of multiplication being 4). As regards length of axial and lateral shoots, significantly higher values were recorded for shoots originated from the dehydration and correspondent cryopreservation treatments in V cryo-plate than in D cryo-plate protocol. Monitoring of rooting parameters also revealed significant variations among the shoots originating from different treatments (Table 2). In general, with few exceptions, in the third subculture after regrowth, rooting ability, particularly the rooting rate, was similar or even greater in shoots originating from all types of

control and cryopreserved explants in comparison with those originating from pregrowth and loading controls. Among shoots regenerated from cryopreserved shoot tips the highest rooting rate (100%) was noticed for those dehydrated with PVS A3 in V cryo-plate protocol. Contrary to these results, Pawłowska and Szewczyk-Taranek (2015) did not find significant differences in multiplication and rooting ability between plants regenerated from control and cryopreseved explants of rosa species.

Table 2. Multiplication (third subculture) and rooting (fourth subculture) of Gisela 5 shoots of different origin after regrowth

Shoot origin	Multiplication parameters			Rooting parameters			
	MI	Length of axial shoot (mm)	Length of lateral shoots (mm)	Rooting rate (%)	No. of roots	Root length (mm)	Shoot length (mm)
Pregrowth control	2.1 f	14.1 bc	5.7 f	75.6 b	5.3 cd	29.0 f	24.2 a
Loading control	2.9 d	14.4 b	7.8 a	68.9 bc	5.9 b	36.0 ab	15.5 cde
PVS A3 40 min-LN	3.7 b	14.2 bc	7.1 bc	75.6 b	4.9 de	33.8 abcd	23.9 a
PVS A3 40 min+LN	4.0 a	15.8 a	7.6 a	100.0 a	4.6 ef	31.3 cdef	18.1 c
PVS3 60 min-LN	3.3 c	13.5 bcd	7.4 ab	73.3 bc	4.6 ef	33.4 bcde	15.3 de
PVS3 60 min+LN	2.9 d	16.3 a	7.7 a	62.2 c	4.8 de	28.0 fg	16.8 cde
2 h desiccation-LN	3.0 d	12.8 cde	5.7 f	63.5 c	4.4 ef	34.7 abc	15.0 de
2 h desiccation+LN	2.5 e	11.9 e	6.0 ef	76.7 b	7.0 a	34.6 abc	17.5 cd
2.5 h desiccation-LN	2.5 e	11.7 e	6.8 cd	80.0 b	3.9 g	37.4 a	14.6 e
2.5 h desiccation+LN	2.6 e	13.6 bcd	6.5 de	80.0 b	4.5 ef	29.7 def	17.3 cd
3 h desiccation-LN	2.9 d	12.2 de	6.3 ef	76.7 b	4.2 fg	25.0 g	20.7 b
3 h desiccation+LN	2.5 e	12.3 de	6.2 ef	76.7 b	5.6 bc	30.0 def	17.6 cd

Mean values of all analyzed parameters within each column followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($P < 0.05$); MI – multiplication index.

Conclusions

In conclusion, we showed that cryopreservation methods using aluminium cryo-plates, especially V cryo-plate method, markedly improve cryostorage success of *in vitro* shoot tips of Gisela 5 rootstock. After marginal modifications of the procedure, D cryo-plate method could efficiently complement the V cryo-plate method. Also, these procedures are simple and user-friendly and can be performed by semi-skilled operators with limited experience in cryopreservation. However, before their large scale application can be foreseen, the optimized protocols should be validated through their application to a range of *Prunus* genotypes.

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THE STATE AND PRODUCTION OF GRASSLANDS IN SERBIA

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Abstract

Given that Serbia (without Kosovo) has large areas under grassland, the aim of this study was to analyze the variation and trend of the areas, total production and yields over 71 years (1948-2018) and prognosis of yield in the next 30 years. The results indicated that the area of grasslands in Serbia declined over the last decade. Most grasslands in hilly-mountain region were degraded and without application of agrotechnical - remedial measures so that large areas were lost. In Autonomous Province of Vojvodina, areas of grasslands have been declining because have been converted into arable land. Total production of meadows increased while total production of pastures decreased during the observed period. The grasslands areas in Serbia showed low yields. In the Vojvodina region, meadows showed decrease in forage productivity, while pastures showed increase in forage productivity. Areas under meadows and pastures should be recultivated and brought into production in accordance with the needs of livestock development. This will result in higher quantity and higher quality production of green mass and hay. Unfortunately, depopulation and under-representation of animals per unit area represented a limiting factor for grassland exploitation. On the other hand, in the future yield growth rates of grasslands in Serbia and Central Serbia and pastures in Vojvodina will very low increase. Contrary, yields growth rates of meadows in Vojvodina will decrease by 2050.

Keywords: *Grasslands, Yield changes, Trend analysis, Serbia.*

Introduction

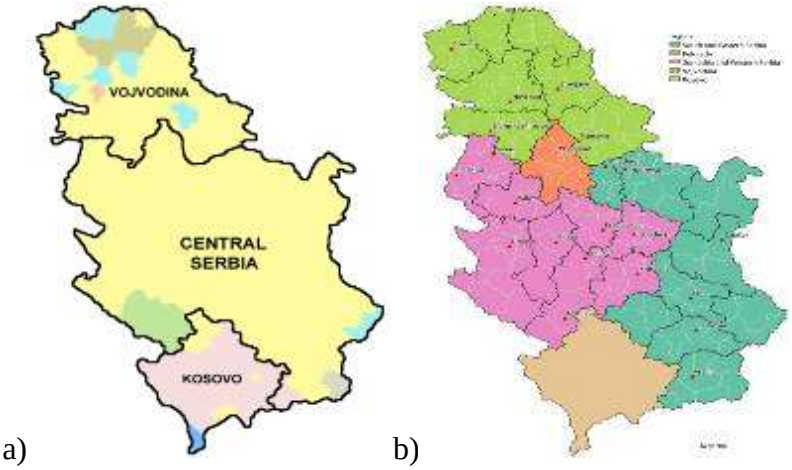
One-third of Earth's land surface is under grasslands (Lemaire *et al.*, 2011). Grasslands are the important feed source for livestock and offer great potential for promoting livestock production sustainability. The livestock raised on semi-natural meadows and pastures has better organoleptic and nutritional qualities of the product (Coulon *et al.*, 2004; Hopkins, 2009). Likewise, grasslands represent a significant source of food for wild animals. They protect soil from erosion, maintain biodiversity and genetic variability within species and contribute to landscape conservation (Đorđević-Milošević *et al.*, 1997; Pärtel *et al.*, 2005). Also, grasslands are vital to supply of water, regulation of flow, carbon storage and climate change mitigation (Bengtsson *et al.*, 2019). In Serbia, grasslands cover a large area. In 2018, grasslands participated with 19.4% in the total utilized agricultural area (meadows participate with 10.1% and pastures with 9.3%) (Statistical Yearbook of the Republic of Serbia, 2019). They are characterized by a rich vegetation and floristic composition. Kojić *et al.* (2004) described 273 grasslands communities in Serbia, while Stošić and Lazarević (2007) pointed out that out of 48 grasslands associations in Serbia, only 10 were economically significant. The share of good-quality species of grasses and legumes in associations directly affect the production and quality of biomass for livestock production. Basically, their share in the natural grasslands of Serbia is small (Tomić *et al.*, 2009; Simić and Vučković, 2014), but can be improved by NPK fertilization, especially with nitrogen (Djuric *et al.*, 2007; Stević *et al.*, 2011; Ivaniš *et al.*, 2013; Zornić *et al.*, 2018; Simić *et al.*, 2019). High quality and

inexpensive forage production on grasslands should be the cornerstone of any cost-effective livestock production. However, in Serbia, the forage production potential of grasslands is not being utilized. Livestock production as a whole is underdeveloped due to low productivity and under-representation of animals per unit of area, especially in hilly-mountainous region of Serbia. Likewise, sparse population and depopulation with a pronounced trend of demographic extinction are an objective constraint on development of livestock and exploitation of grasslands.

The study analyses the area, production and yield change of meadows and pastures in Serbia during the period 1948 to 2018. Also, in this study we make a forecast of future yield trends based on historical data obtained from the Statistical office of the Republic of Serbia.

Material and Methods

The data collected from the Agriculture in Serbia, 1947-1996 (1998) and Statistical Yearbook of the Republic of Serbia from 1997 to 2019 were used, referring to the area, production and yield of meadows and pastures of the Republic of Serbia (without Kosovo), Central Serbia and Vojvodina. Central Serbia includes following regions: South and Eastern Serbia, Belgrade and Šumadija and Western Serbia (Picture 1; source: a) https://es.m.wikipedia.org/wiki/Archivo:Serbia_ethnic02.png and b) http://www.lokalni-razvoj.org/upload/Book/Document/2012_06/Inter_Newsletter_1_English.pdf



a) b)

Picture 1. Republic of Serbia (a) and regions of Serbia (b).

Analytical and comparative methods were used in this paper. The mean area, total production and yield as well as their coefficient of variations and trends were calculated. Trend determined the tendency of time series data to increase or decrease over time. The linear least squares regression was used to predict trends in data ($y = ax + b$; y - area, total production or yield; a and b - parameters of the model). The Excel was used for statistical data analysis.

Results and Discussion

According to Table 1, the area of meadows in Serbia ranged between 321812 ha (2017) to 670685 ha (1984), with a mean of 592182.8 ha and coefficient of variation 13.54%, during the 71-year period. The total production of meadows in Serbia ranged between 121060 t (1974) to 1334770 t (1984), with a mean of 975681.1 t and coefficient of variation 25.62%, during the 71-year period. The average annual yield of meadows in Serbia ranged between 0.955 t ha^{-1}

(1952) to 2.3 t ha^{-1} (2016) with a mean of 1.70 t ha^{-1} and coefficient of variation 17.2%, during the 71-year period. The average annual yield of meadows and coefficient of variation in Vojvodina were higher by 16.7 % (1.94 t ha^{-1}) and 11.6% (21.10%) than in Central Serbia (1.66 t ha^{-1} and 18.09%, respectively) during this period.

Table 1. Descriptive statistics for area (ha), total production - TP (t) and yield (t ha^{-1}) of meadows in Serbia, Central Serbia and Vojvodina from 1948 to 2018.

Region	Item	Mean	Minimum	Maximum	CV, %
Serbia	Area	592182.8	321812	670685	13.54
	TP	975681.1	121060	1334770	25.62
	Yield	1.70	0.955	2.3	17.20
Central Serbia	Area	501593	305903	575670	12.45
	TP	834821.7	412030	1137009	21.95
	Yield	1.66	0.924	2.3	18.09
Vojvodina	Area	37248.9	15909	57805	21.03
	TP	72830.6	19820	153850	34.21
	Yield	1.94	1.02	2.888	21.10

CV – coefficient of variation (%)

As for pastures, the average area in Serbia ranged between 294622 ha (2017) to 1061690 ha (1959), with a mean of 863855.1 ha and coefficient of variation 23.80%, from 1948 to 2018 (Table 2). For this period, the total production ranged between 262000 t (2000) to 825280 t (1959), with a mean of 488378.2 t and coefficient of variation 20.6%. The average annual yield in Serbia ranged between 0.326 t ha^{-1} (2000) and 2.0 t ha^{-1} (2018) with a mean of 0.62 t ha^{-1} and coefficient of variation 49.5%. The average annual yield of pastures in Vojvodina (1.09 t ha^{-1}) was higher than in Central Serbia (0.546 t ha^{-1}) with a lower coefficient of variation (33.07% and 61.72%, respectively) during an investigated time period. The significantly higher mean yields of grasslands in the Vojvodina are the result of favourable growing conditions for the plants. Reasons for better plants growth include more favourable soil properties (deeper soils with better structure and chemistry) (Ćirić *et al.*, 2012) compared to shallow mountain soils with low productivity (Simić *et al.*, 2015).

Table 2. Descriptive statistics for area (ha), total production - TP (t) and yield (t ha^{-1}) of pastures in Serbia, Central Serbia and Vojvodina from 1948 to 2018.

Region	Item	Mean	Minimum	Maximum	CV, %
Serbia	Area	863855.1	294622	1061690	23.80
	TP	488378.2	262000	825280	20.60
	Yield	0.62	0.326	2.0	49.50
Central Serbia	Area	609891.5	226277	724569	22.84
	TP	293214.1	153590	504911	22.32
	Yield	0.546	0.262	2.0	61.72
Vojvodina	Area	127801.2	41554	186626	24.16
	TP	137897	55841	310840	37.89
	Yield	1.09	0.5	2.318	33.07

CV – coefficient of variation (%)

The regions showed the high coefficient of variation for all parameters of meadows and pastures, especially for yield, indicating a heterogeneous environment. In general, the yield oscillated with high amplitude. The yield, therefore, has fluctuated from year to year,

sometimes vary sharply. The high grassland yield stability must be the primary goal of livestock farmers. However, according to Li *et al.* (2011), the grassland biomass is significantly correlated to the rainfall. Grassland yield is influenced by total amount of annual rainfall and its frequency and intensity (Zhai *et al.*, 2005). The lower amount of rainfall and higher temperatures at the summer months caused the mowing of grasslands at only once a year. In addition, permanent grasslands in Serbia have lower yields because they are spread on low productivity soils (Simić *et al.*, 2015). The soil nutrients deficiencies and the habitat moisture are main factors determined development of grasslands in Serbia (Aćić *et al.*, 2013). Also, the grassland has sub-optimal botanical composition with a low proportion of perennial legumes from 6.73% to 34.12%, low yields and poor quality of forage (Djuric *et al.*, 2007). Djukic *et al.* (2008) pointed that the forage yield and quality of grasslands depend on the floristic composition, the soil type and climatic factors (rainfall, temperature and light). Also, low and unstable yields of natural grasslands are the result of the absence of application of agro-technical measures (Dubljević, 2007). The application of mineral fertilizer, especially nitrogen could significantly increase forage yield and quality of grasslands and consequently, the animal production (Delevatti *et al.*, 2019). In general, nitrogen is the basis for high yields of natural grasslands (You *et al.*, 2017). Tomić *et al.* (2018) concluded that the optimum nitrogen level for forage yield of natural grassland of the *Arrhenatheretalia* order on the Kopaonik is 80 kg ha^{-1}. Stošić and Lazarević (2007) concluded that the ratio N: P_2O_5: K_2O should be 2-3: 1: 1 and N: P: K - 5-9: 1: 2, respectively. However, nitrogen fertilizer should be applied on grasslands at altitudes up to 1000 m a.s.l., because regenerative ability significantly decreases on grassland above 1000 m a.s.l. so it is not economically viable (Lazarević *et al.*, 2004). Also, cattle manure as fertilizer can greatly improve grassland productivity, increase the proportion of legumes and crude protein contents of the grasslands (Nemera *et al.*, 2017; Simić *et al.*, 2019). The agrotechnical measures which should be implemented to improve grassland productivity are drainage, irrigation and landscape management, levelling, dragging, rolling and land clearing (destruction of bushes, deforestation, removal of stumps and stones). On the other hand, the undersowing is not recommended for use on grasslands in Serbia (Ćupina *et al.*, 2005). The reason is that grasslands in Serbia are distributed on shallow, dry and scaffold soil, which makes it difficult to apply this measure. Similar, Huguenin-Elie *et al.* (2006) reported that undersowing of legumes in grasslands had no effects on forage yield and quality.

Results in Figure 1-3 showed that the area and total production of meadows in Serbia and regions declined sharply in the last decade. So, these investigated parameters showed the similar tendency. Also, the yield of meadows in Serbia and Central Serbia had similar tendency of increase by 5.1 and 6.4 kg ha^{-1}, respectively (Figure 4). However, the yield of meadows in Vojvodina decreased by 8.2 kg ha^{-1}.

The area of pastures in Serbia declined after year 1998, in Central Serbia in the last decade (Figures 5 and 6). The total production of pastures in Serbia declined by 1648.2 t, while total production of pastures in Central Serbia increased by 1247.7 t. The area and total production of pastures in Vojvodina declined by 1412.2 ha and 1669.4 t, respectively (Figure 7). The yield of pastures in Serbia, Central Serbia and Vojvodina have slight increase tendency by 4.8, 7.2 and 0.7 kg ha^{-1}, respectively (Figure 8).

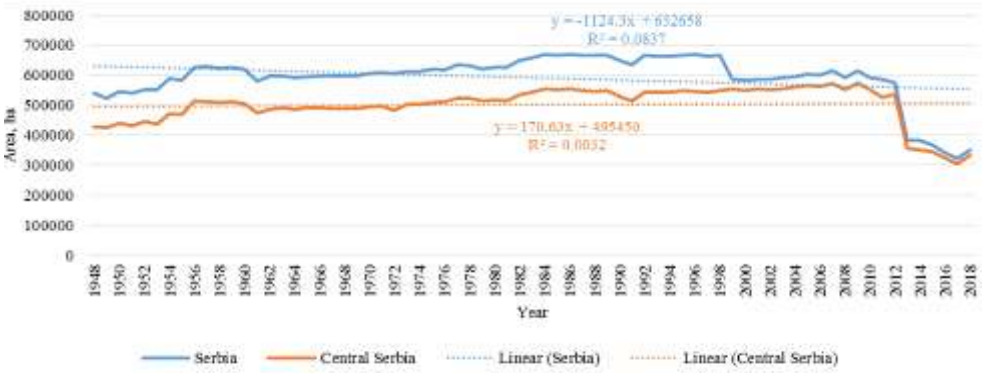


Figure 1. The area of meadows in Serbia and Central Serbia during 1948 – 2018.

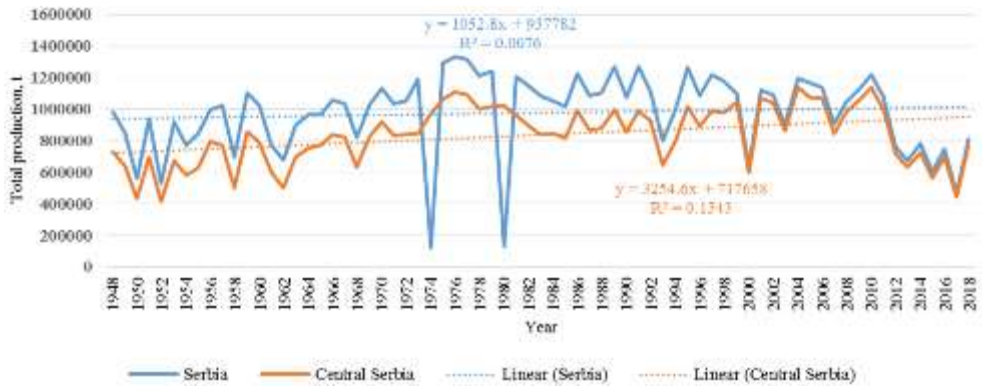


Figure 2. Total production of meadows in Serbia and Central Serbia during 1948 – 2018.

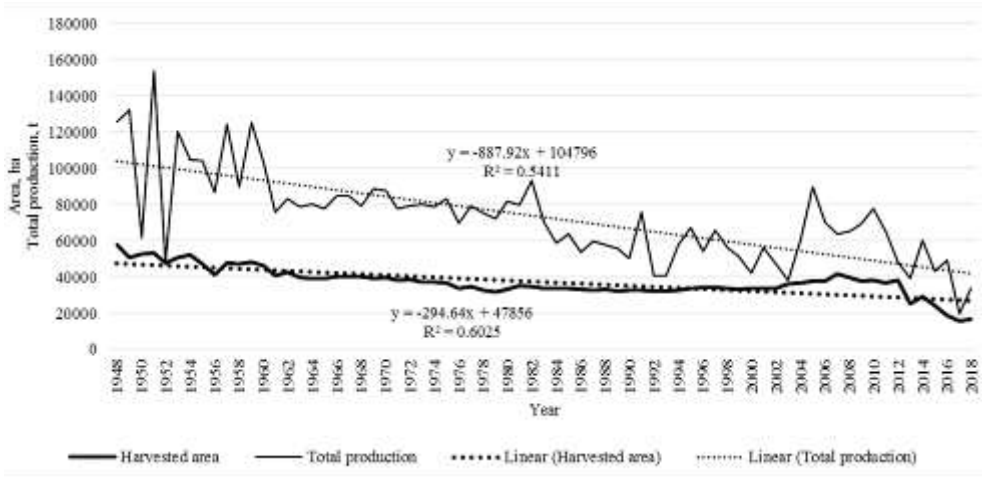


Figure 3. The area and total production of meadows in Vojvodina during 1948 – 2018.

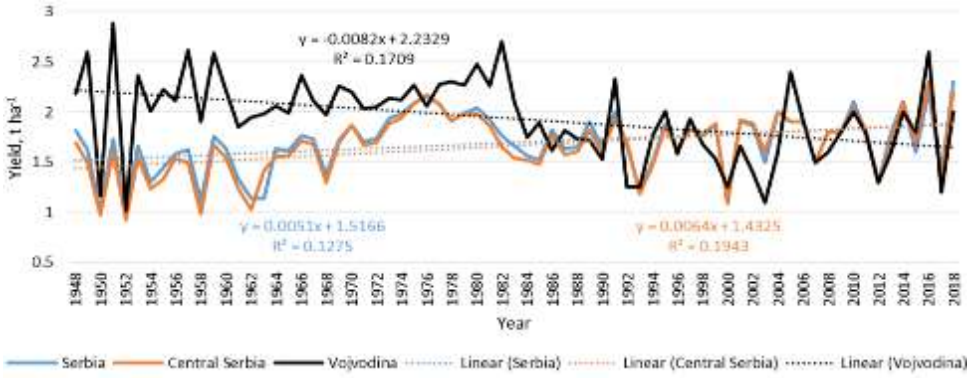


Figure 4. Yield of meadows in Serbia, Central Serbia and Vojvodina during 1948 – 2018.

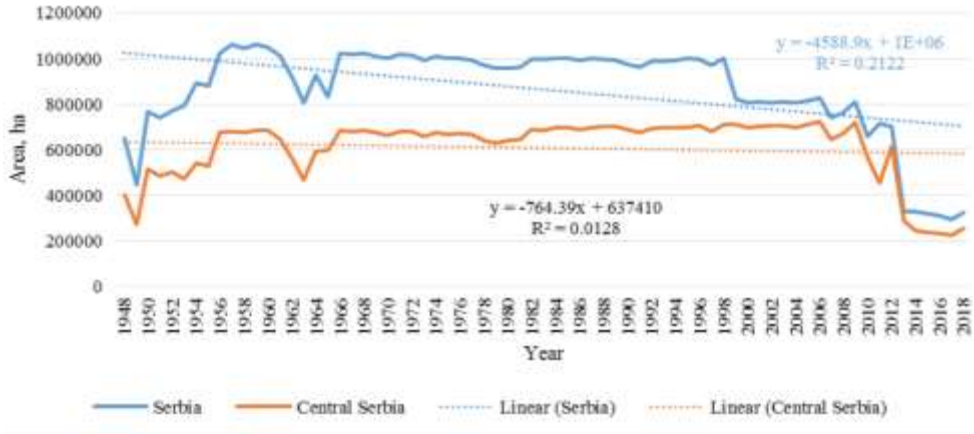


Figure 5. The area of pastures Serbia and Central Serbia during 1948 – 2018.

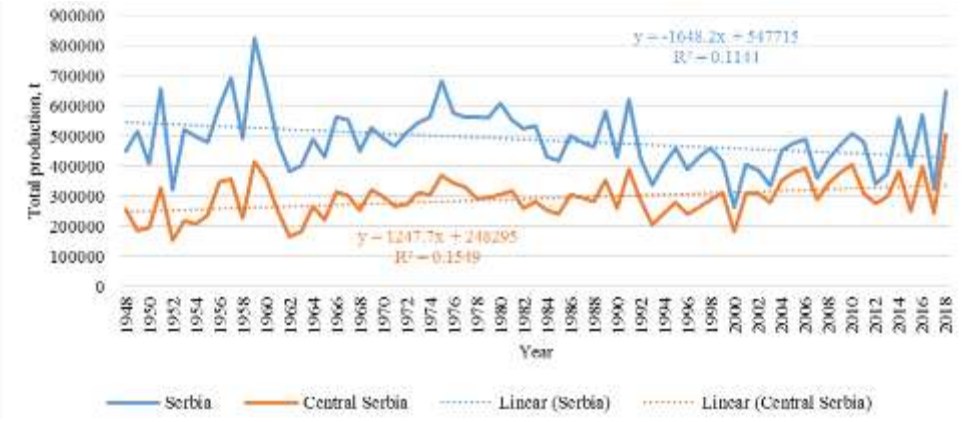


Figure 6. Total production of pastures in Serbia and Central Serbia during 1948 – 2018.

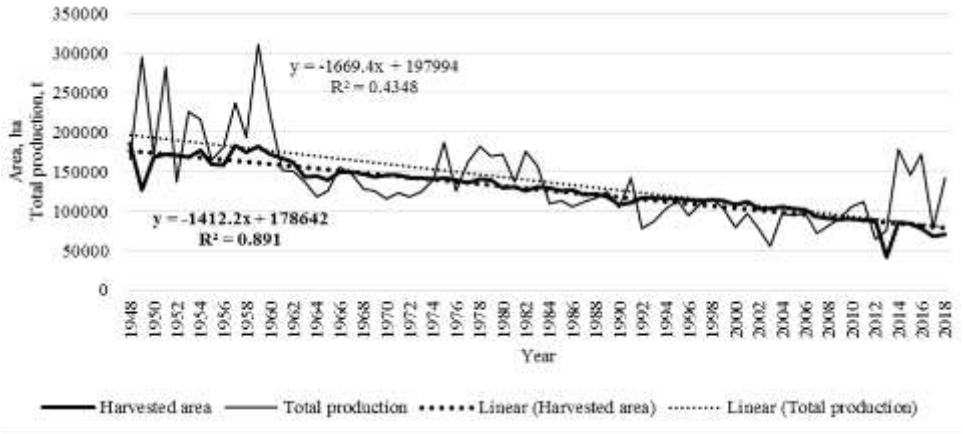


Figure 7. The area and total production of pastures in Vojvodina during 1948 – 2018.

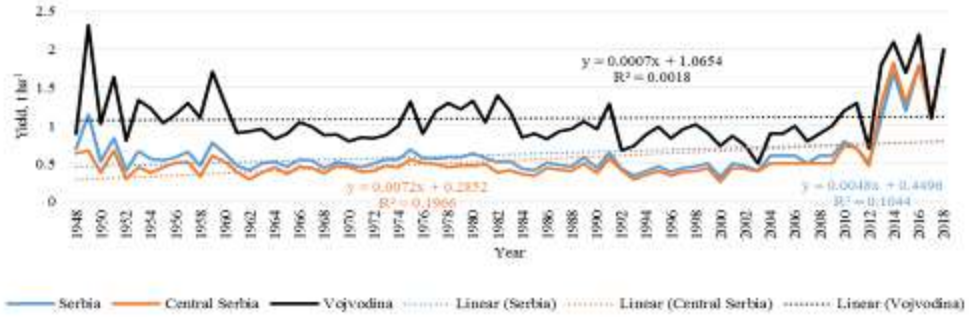


Figure 8. Yield of pastures in Serbia, Central Serbia and Vojvodina during 1948 – 2018.

In general, area of grasslands decreased due to the decline of ruminant population in Serbia. Unfortunately, without livestock development, permanent meadows and pastures will continue to be inadequately used. Similar to our results, Egoh *et al.* (2016) find that the grasslands worldwide have been in drastic decline during the last decades. This phenomenon is the result of conversion to arable land for production of animal feed crops and lack of management and abandonment (Queiroz *et al.*, 2014).

Basically, the production potential of better natural grasslands and their quality can be increased by the application of agrotechnical measures, especially fertilization and weed control. Also, through the planned utilization and cultivation of pastures, the floristic composition would be improved and preserved.

Essentially, the interest in improving the production of meadows and pastures has decreased due to the reduction in the number of ruminants (cattle, sheep and goat). For that reason the grasslands are increasingly degraded and reduced and in more remote areas they are under extreme degradation and shrub vegetation develops. Such surfaces are further gradually excluded from the production cycle. The large increasing production and supply of livestock breeding, as well as agricultural policy developments are essential for grassland farming system and sustainable agricultural development.

The time series analysis predicts the future by projecting the historical data. Therefore our forecast is based on random trends and relationships existing in the historical data from 1948 to 2018. We find that yields of grasslands in Serbia and Central Serbia and yield of pastures in Vojvodina will increase by 2050 (Figure 9). However, these are very low yield growth rates. Contrary, yields of meadows in Vojvodina will decrease by 2050. Essentiality, there is almost no or little agronomic input in the grasslands of Serbia, so there are no technological

improvements in dry matter productivity. Contrary, Qi *et al.* (2018) estimated that dry matter yield of grasslands in the United Kingdom will decrease by about 2.5 to 5% from 2020s to 2050s.

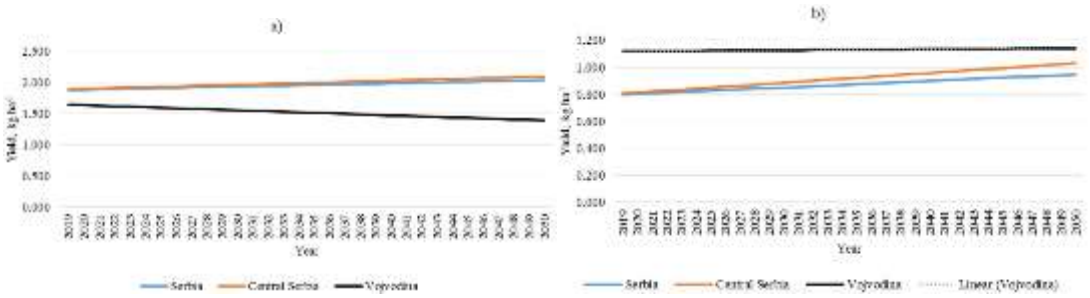


Figure 9. Yield prognosis for meadows (a) and pastures (b) in Serbia, Central Serbia and Vojvodina.

Conclusions

The study emerges as an important information tool in understanding the change in the area, production, and yield of Serbian grasslands, with particular reference to factors and problems which influence high and stable production. Serbia has large areas under grasslands. They should be a major feed production resource. These grassland forage resources should be brought to a much higher level of productivity and quality in order to improve the productivity and quality of livestock. The forecast shows the grassland production having a weak upward trend. In general, areas under meadows and pastures should be recultivated and brought into production in accordance with the needs of livestock development. This will result in higher quantity and higher quality production of green mass and hay. Our study needs to be further refined and take into account climate change, rising carbon dioxide emissions in the atmosphere and technological advances based on annual yield.

Acknowledgment

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PROPAGATION OF SPIRAEA ×VANHOUTTEI (BRIOT) CARRIÈRE BY SOFTWOOD, SEMIHARDWOOD AND HARDWOOD CUTTINGS

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Abstract

Van Houtte's spiraea is low maintenance ornamental plant that is considered highly resistant to urban pollution, and it also tolerates moderate drought. The bridalwreath spiraea can be propagated by cuttings, but there is no sufficient data about expected rooting rate for different cutting types. Therefore, we decided to investigate rooting success of softwood, semi-hardwood and hardwood cuttings of bridalwreath spirea thus establishing the optimal method for its vegetative propagation. The group of *S. × vanhouttei* vigorous and healthy shrubs, 8 years old was selected as cuttings source. Green cuttings were taken in July, semi-hardwood cuttings were taken in September, and both cutting types were treated with dust preparation of 0.1% IBA before sticking in sand. Hardwood cuttings were taken in January, the base of cuttings was wounded 1 cm deep, and also treated with 0.1% IBA. Obtained results showed that there were considerable differences in a rooting of these cutting types. The best results were obtained using hardwood cuttings (93.3% rooted cuttings) followed by semi-hardwood cuttings (85.3% rooted cuttings). However, there was no statistically significant difference between cuttings treated with 0.1% IBA and the control treatment; rooting percentage was slightly lower in a control (91% rooted hardwood cuttings, 83% rooted semi-hardwood cuttings), indicating that auxine treatment could be omitted during vegetative propagation of *S. × vanhouttei.*

Key words: *Van Houtte's spiraea, hardwood cuttings, semi-hardwood cuttings, green cuttings, vegetative propagation.*

Introduction

The bridalwreath spirea (*Spiraea × vanhouttei* (Briot) Carrière, fam. Rosaceae) is a deciduous shrub, up to 2.5 m tall, with arching, graceful, fountain-like branches. It is valued for its showy spring bloom. The flowers are white, numerous, densely covering the branches, often visited by butterflies. The leaves are bluish-green. Van Houtte's spiraea is low maintenance ornamental plant that can be easily grown in well-drained soils in full sun. It is also considered highly resistant to urban pollution, and tolerates moderate drought. It is often used as a decorative shrub in green spaces in Serbia, usually as a hedge, border, woodland margins, accent or screen plant (Pavlovic *et al.*, 2007; Ingels, 2009). The bridalwreath spirea (*Spiraea × vanhouttei* (Briot) Carrière, fam. Rosaceae) is a deciduous shrub, up to 2.5 m tall, with arching, graceful, fountain-like branches. It is valued for its showy spring bloom. The flowers are white, numerous, densely covering the branches, often visited by butterflies. The leaves are bluish-green. Van Houtte's spiraea is low maintenance ornamental plant that can be easily grown in well-drained soils in full sun. It is also considered highly resistant to urban pollution, and tolerates moderate drought. It is often used as a decorative shrub in green spaces in Serbia, usually as a hedge, border, woodland margins, accent or screen plant (Pavlovic *et al.*, 2007; Ingels, 2009).

The Van Houtte's spiraea is usually mass-propagated by cuttings (Hartmann *et al.*, 2014). Although some authors state that *S.* × *vanhouttei* can be succesfully propagated by softwood cuttings (Macdonald, 1996; Lamb *et al.*, 1975), there is no sufficient data about expected rooting rate. Besides, Hartmann *et al.*, (2014) indicate that spireas can also be propagated by semi-hardwood or hardwood cuttings. Therefore, authors decided to investigate rooting success of softwood, semi-hardwood and hardwood cuttings of bridalwreath spirea thus establishing the optimal method for its vegetative propagation.

Material and Methods

The group of *S.* × *vanhouttei* vigorous and healthy shrubs, 8 years old was selected as cuttings source. The green cuttings 12-16 cm long, with 8-11 nodes and diameter of 2-3 mm, were taken on July, 20, 2017. Basal leaves were removed leaving only 4-6 leaves on top of cuttings. The base of cuttings (1 cm long) was treated with dust preparation of 0.1% IBA before deeply sticking in sand, to the remaining leaves. Four replications of 25 cuttings were used per treatment. The cuttings were covered with polyethylene film to maintain humidity. Watering was performed using a hose The rooting was performed in high tunnel greenhouse where temperature and air humidity were recorded regularly. The state of cutings and rooting percentage were determined 4 weeks after sticking. The semi-hardwood cuttings, mostly without leaves, were taken on September, 25, in 2017. The cuttings were 10-15 cm long, with 2-4 mm diameter, containing 7-10 nodes. They were treated with dust preparation of 0.1% IBA and inserted to a depth of 10 cm into the same substrate as for the green cuttings, in high tunnel greenhouse. Four replications of 50 cuttings were used both treated with auxine and in the control treatment. The rooting percentage and state of cuttings were recorded on May, 15 of the following year. The hardwood cuttings, 10-15 cm long, with 2-4 mm diameter, containing 7-10 nodes, were taken on January, 10, 2018. The base of cuttings was wounded 1 cm deep, using a sharp knife, and treated with dust preparation of 0.1% IBA. Four replications of 50 cuttings were used per treatment. The cuttings were kept in sand, placed in inverted position with distal part on the bottom, with the aim to delay bud proliferation. On April, 2, the cuttings were taken and placed vertically into the rooting substrate (sand). The rooting percentage and state of cuttings were recorded on August, 15. In the control the auxine treatment was omitted for all cutting types. After sticking, substrate and cuttings were treated with fungicide Ridomil, Burgundy mixture and Sistemin insecticide and acaricide. The significance of differences between the means was determined by the analysis of variance (ANOVA, $p < 0.05$) and the least significant difference (LSD) test.

Results and Discussion

The rooting of the green cuttings was performed in a tunnel greenhouse without automatic control of humidity and temperature. Humidity level ranged between 42.3% and 95% and temperature oscillated between 21.1°C and 37.8°C. For this reason, cuttings lost their leaves and only one cutting treated with auxine (1%) and one cutting in a control treatment (1%) were rooted. The rooting of semi-hardwood cuttings was done in the same greenhouse, where temperature ranged from 9.5°C to 32.1°C, and humidity level ranged from 41.4% to 95% during October. However, these cuttings were mostly without leaves in the time of sticking, so low humidity didn't affect their survival. They overwintered in this tunnel greenhouse without heating, but temperature didn't drop below 0°C during winter. The buds proliferated in April, and majority of shoots reached length of 30 cm in May. The rooting percentage of cuttings treated with 0.1% IBA was relatively high (85.3%), but difference between cuttings treated with 0.1% IBA and the control treatment was not statistically significant (Table 1).

Table 1. Rooting percentage of semi-hardwood cutting

Treatment	Min	Max	$\bar{X}$
0.1% IBA	83.0	91.0	85.3[a]
Control	81.0	85.0	83.0[a]

Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

The rooting of hardwood cuttings was the most successful, with rooting percentage of 95 % (Table 2), while majority of shoots reached length of 15 cm. The cuttings in a control treatment rooted also in a high number (91%) indicating that auxine treatment can be omitted during propagation of *S. × vanhouttei.*

Table 2. Rooting percentage of hardwood cutting

Treatment	Min	Max	$\bar{X}$
0.1% IBA	91.0	95.0	93.3[a]
Control	90.0	92.0	91.0[a]

Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

Besides *S. × vanhouttei,* some other spireas can also be successfully propagated using hardwood cuttings. For example, propagation using hardwood cuttings is recommended for *S. douglasii* with expected rooting rate of 90% - 100%, without any pretreatment (Darris, 2002). Results obtained during our reserach showed that use of mist or fogging system is necessary during rooting of *S. × vanhouttei* green cuttings to prevent desiccation. However, Aiello and Graves (1998) showed that use of subirrigation method instead of intermittent mist or fogging can be effective in rooting of green cuttings of *Spiraea × bumalda* Burv. 'Goldflame', with rooting rate higher than 90%. They considered that although intermittent mist or fogging systems are necessary to prevent leaf desiccation, these methods also increase production costs and can leach nutrients from foliage. In spite of that, research conducted by Aiello and Graves (1998), showed that use of the subirrigation methods without mist is not suitable for rooting of green cuttings of other woody plants, such as *Prunus serrulata* 'Kwanzan' or *Syringa vulgaris* 'Michael Buchner'. High rooting level of *S. × vanhouttei* green cuttings without use of mist system was recorded by Hansen and Kristiansen (2000). They researched the effect of cutting taking date on root formation of several woody shrubs including *S. × vanhouttei*. Cuttings were taken from July to October and they were planted directly in the field and covered with polyethylene. Obtained results showed that cuttings of *S. × vanhouttei* taken til mid August had higher rooting percentage than cuttings taken from mid August to October, thus indicating that rooting of green cuttings is better than semi-hardwood cuttings, despite the lack of mist system. This difference can be explained by different climate conditions during our experiment and experiment conducted by Hansen and Kristiansen (2000). Although the rooting of green cuttings in our research was low, the results obtained with hardwood cuttings are very satisfactory, and this method can be used for efficient propagation of *S. × vanhouttei* in Serbia.

Conclusions

The most suitable and cost-effective vegetative method for propagation of *S. × vanhouttei* in Serbia is by hardwood cuttings, with expected rooting rate of more than 90%. Satisfactory results can be obtained using semi-hardwood cuttings, with expected rooting rate of more than 80%.

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THE INFLUENCE OF DIFFERENT SEED TREATMENTS ON DORMANCY BREAKING AND GERMINATION OF LIRIODENDRON TULIPIFERA L.

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Abstract

Liriodendron tulipifera is a large, deciduous tree up to 50 m tall. It is admired for its tulip-like, melliferous flowers that bloom in spring. Tulip tree is also a good shade tree and its wood is used for furniture and general lumber. Recently, it became very popular as an ornamental tree in Serbia and we decided to research a possibility of generative propagation of selected two elite trees growing in the Belgrade area. During the experiment, the seeds collected in October were cold stratified in sand or in bags without a substrate, for 2, 3 or 4 months. Obtained results showed that quality of seeds was low with a small number of viable seeds. Germination rate did not exceed 16%, but the real germination rate (calculated as a percentage of sound seeds that germinate) was considerably higher, reaching 82.5%. Besides, considerable difference in a response to stratification method between seeds collected from different trees was recorded. Seeds collected from one of the mother trees did not show significant difference in real germination rate after 2 or 3 months stratification in sand (66.5% and 64.6%, respectively), but seeds collected from the other tree had higher real germination rate after being stratified for 3 months in sand (82.5%) than after 2 months of stratification in sand (only 18.8%). Although genotype strongly influenced obtained results, we can conclude that 3 months stratification in sand showed overall the best results, but seed viability should be tested before its use for propagation.

Key words: *tulip tree, seed dormancy, cold stratification, generative propagation.*

Introduction

Tulip poplar (*Liriodendron tulipifera* L., Fam. Magnoliaceae) is a large, deciduous tree up to 50 m tall. The leaves are four-lobed, bright green becoming golden yellow in autumn. It is admired for its large, tulip-like, melliferous flowers, yellow with an orange band at the base of each tepal. Tulip poplar blooms in spring, and cone-shaped, dry, scaly fruits are ripen in autumn, bearing numerous winged seeds. Tulip tree is a fast-growing species, which needs full sun and organically rich, well-drained soil. It forms conical, dense canopy which provides very good shade. Its wood is used for furniture, plywood, veneer and general lumber. (Bonner and Karrfalt, 2008; Vukićević, 1982).

It has been grown as an ornamental tree in Serbia for decades, but recently, it became very popular and it is widely planted. The common propagation method is by seeds (Hartmann *et al.*, 2014; Bonner and Karrfalt, 2008), although it can be vegetatively propagated using green cuttings (Enright, 1957; Bin *et al.*, 2018), root cuttings or grafting (Hartmann *et al.*, 2014). Many researchers reported successful *in vitro* propagation of tulip tree, using mainly somatic embryogenesis method (Merkle and Sommer, 1986; Lee *et al.*, 2003; Kim *et al.*, 2016), but it is not used in a commercial propagation of this species.

L. tulipifera seeds has embryo dormancy and cold stratification is required for seed germination. However, recommended pretreatments are different, and some authors advise cold, moist stratification in plastic bags for 60 or 90 days at the temperature 2 to 5 °C, while

others suggest alternating weekly temperatures of 0 and 10 °C or 2 and 12 °C (Regent, 1980; Bonner and Russell, 1974; Boyce and Hosner, 1963; Hartmann *et al.*, 2014). The use of peat moss or sand as a stratification media is also recommended (Bonner and Karrfalt, 2008)
For this reason, authors decided to select elite tulip trees grown in Belgrade in Serbia, that are well adapted to conditions in Belgrade and to investigate an effect of different pregermination treatments on seed germination.

Material and Methods

The two tulip trees planted next to each other were selected as a seed source. The fruits were collected in October, 2018., brought in the Laboratory for seed testing at the Faculty of Forestry, University of Belgrade (Serbia), and spread out to dry at the room temperature. The dried cones were broken apart by hand and extracted seeds were used for experiments. The seeds were treated with Previcur N fungicide (0.3 % v/v) and following pretreatments were used:

- cold stratification in sand
The seeds were stratified in sand (0.75-1.00 mm) at the temperature 3 - 5ºC, for 2 and 3 months. Moisture was controled regularly and distilled water was added if necessary.

- cold stratification in plastic bags at constant temperature
The seeds were stratified in plastic transparent bags (naked stratification) at the temperature 3 - 5ºC, for 2 and 3 months. Bags were opened regularly and moisture was controled.

- cold stratification in plastic bags at alternating temperatures
The seeds were stratified in plastic transparent bags (naked stratification) at the temperatures 3ºC and 12 ºC that were alternating each week. The moisture was controled regularly, seeds were rinsed using a cold water once a week, and stratification duration was 3 and 4 months.

The seeds originating from different trees were separated in order to determine the influence of genotype on germination. After stratification, the seeds were placed on the top of two layers of filter paper in the petri dishes for germination. Temperature was 20 - 23°C and humidity was controlled daily by adding the distilled water. According to ISTA rules (International Seed Testing Association, 1996), first count (germination energy) was recorded on 7th day, after that the number of germinated seeds was recorded daily, and the final count was noted on 28th day. After that, the seeds were cut with the aim to determine the percentage of viable seeds which is needed to calculate the real germination rate, as a percentage of sound (viable) seeds that germinate (Grbić *et al.*, 2010).
Each treatment consisted of four replicates with 50 seeds. Obtained data were statistically analysed using the program Statgraphics Plus, Ver 2.1.

Results and Discussion

Obtained results showed that germination was very low (Table 1). The highest germination rate was recorded after 3 months of stratification in sand or in bags: 16% and 14.5% for tree number 1 and 6% and 9% for tree number 2. However, low germination rate not exceeding 8% has already been recorded for tulip tree (Sulyga, 2015; Keun-Ok *et al.*, 2009). In spite of that, the real germination rate (percentage of sound seeds that germinate) was much higher reaching 82.5% (Table 1). This was expected because *L. tulipifera* naturally has low proportion of filled seeds (Bonner and Karrfalt, 2008) and for that reason a cutting the

ungerminated seeds after testing to determine if embryos are present is recommended. For example, Keun-Ok *et al.* (2009) recorded only 27.6% of sound seeds in their research. Viability of seeds can also be evaluated by tetrazolium test (International Seed Testing Association 1996).

Table 1 Germination of collected seeds after different stratification pretreatments

Treatment	Germination rate	Real germination rate	Germination energy
2 months in sand - tree 1	11.3^{ab}	66.5^{b}	1.3^{ab}
3 months in sand - tree 1	16.0^{a}	64.6^{b}	2.0^{ab}
2 months in bag - tree 1	3.0^{de}	49.9^{cd}	0.0^{b}
3 months in bag - tree 1	14.5^{a}	53.5^{cd}	1.0^{ab}
3 months 3 - 12ºC in bags - tree 1	5.3^{cd}	55.5^{bc}	1.3^{ab}
4 months 3 - 12ºC in bags - tree 1	0.0^{e}	0.0^{f}	0.0^{b}
2 months in sand - tree 2	5.5^{cd}	18.8^{ef}	0.5^{ab}
3 months in sand - tree 2	6.0^{cd}	82.5^{a}	2.5^{a}
2 months in bag - tree 2	0.5^{e}	12.5^{ef}	0.0^{b}
3 months in bag - tree 2	9.0^{bc}	42.1^{cde}	1.5^{ab}
3 months 3 - 12ºC in bags - tree 2	3.0^{de}	20.8^{ef}	0.0^{b}
4 months 3 - 12ºC in bags - tree 2	3.0^{de}	25.0^{cdef}	1.0^{ab}

Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

Furthermore, our research showed that germination of seeds originating from tree number 1 and tree number 2 was different depending on treatment used, indicating that genotype probably affected the germination (Table 1). For example, after 2 months stratification in sand the real germination rate for tree number 1 was 66.5%, and for tree number 2 was only 18.8%, but after 3 month stratification in sand the real germination rate was 82.5% for tree number 2 and 64.6% for tree number 1. The influence of genotype on seed germination is well-known fact (Hartmann *et al.,* 2014), but the response of some genotypes on different sowing pretreatments can be same for all genotypes tested (Demirsoy *et al.,* 2010), or some genotypes can respond positively to some treatments while others can show negative response to the same treatments (Acar *et al.,* 2017). Also, differences in seeds response to a different treatments were larger for tree number 2, with a real germination rate ranging from 12.5% to 82.5%, while the real germination rate obtained for tree number 1 ranged mainly between 49.9% and 66.5% (Table 1).

Besides, after 3 months of stratification, seeds collected from tree number 1 had higher germination rate (16%) and lower real germination rate (64.6%) compared to the second mother tree where germination was 6% and real germination rate was 82.5%. This indicates that tree number 1 perhaps produced larger number of sound seeds than tree number 2.

Although some authors (Regent, 1980; Boyce and Hosner, 1963) stated that good results can be obtained by alternating temperatures during stratification, our results didn't prove their statements, and both the germination rate and real germination rate were low compared to the results obtained after other conducted treatments (Table 1).

Conclusions

Obtained results showed that *L. tulipifera* has a small number of viable seeds and for this reason the germination rate did not exceed 16%. However, the real germination rate was considerably higher, reaching 82.5% after 3 months stratification in sand. Genotype strongly influenced obtained results, both the germination rate and the percentage of viable seeds produced. We can conclude that selected elite trees can be used as a seed source for tulip tree propagation, but expected germination rate is low due to the small percentage of sound seeds.

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VARIABILITY FOR KERNEL TOCOPHEROLS AMONG MAIZE INBRED LINES

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Abstract

Maize, among cereals, has the greatest content of tocopherols, which play important role in human and animal health and plant metabolism. In this study content of α, ß+γ and δ tocopherols was measured in 123 maize inbred lines: 74 with standard kernel type, 37 sweet corn and 12 popcorn, aimed to determine inbred lines that could be used in breeding program to improve tocopherols content. Inbred lines with standard kernel type have the highest average content of α tocopherols (14.72 µg/g DW) and sweet corn lines have the highest content of ß+γ tocopherols 72.81 µg/g DW. Popcorn lines have the lowest average content of all tocopherols. ß+γ-tocopherol had the highest level of variation (range: 30.79 to 115.65 µg/g), followed by α-tocopherol (range: 3.12 to 25.47 µg/g,), and δ-tocopherol possessed moderate degree of variation (range: 0.21 to 4.27) µg/g. The content of α-tocopherol ranged from 3.41 to 25.47 µg/g DW in inbreds with standard kernel type and from 4.17 to 19.01 DW in sweet corn inbreds and ß+γ tocopherol content vary from 30.79 to 105.62 µg/g DW in inbreds with standard kernel type and from 42.56 to 115.65 µg/g DW in sweet corn inbreds. Two sweet corn inbred lines with highest content of ß+γ tocopherols were identified: Gold-5 and Esteem 1-13, as well as two lines with standard kernel type with the highest content of α tocopherols TVA973 and inbred H. Inbred lines with high content of α as well as γ tocopherols may be used in breeding programs to improve nutritional value.

Keywords: *maize, nutritional quality, vitamin E, variability*

Introduction

Vitamin E is the term for the group of eight lipid-soluble antioxidants occurring in nature especially in plants. The aforementioned groups are divided in two distinct classes tocopherols and tocotrienols that differ only in the position and number of methyl groups on their chromal ring. The two groups are classified according to the location of the methyl group as α-tocopherol, β-tocopherol, δ-tocopherol, γ-tocopherol, α-tocotrienol, β- tocotrienol, δ-tocotrienol and γ-tocotrienol (Moss, 1981). Vitamin E is important to human health, animal growth, and plant development. Tocopherols play an important role in seed maturation, storage and germination, photo-assimilating transportation, abiotic stress response, cell membranes protection from oxidation, protection of the photosynthetic apparatus, and may affect gene expression (Fryer 1992; Munné-Bosch and Alegre 2002; Sattler *et al.* 2004; Falk and Munné-Bosch 2010; Me`ne-Saffrane´a *et al.* 2010). The roles of tocopherols in humans could be the preservation of membrane integrity from oxidative damage, prevention of Alzheimer's disease, neurological disorders, cancer, cataracts, inflammatory diseases and age-related macular degeneration (Combs, 1992; Stampfer *et al.*, 1993; Sies and Stahl, 1995, Dietrich *et al.* 2006; Muller 2010). For both adults and teenagers 15 mg/day of vitamin E is recommended whereas in children (0– 6 month) recommendation is 4 mg/day (Food and nutrition board, 2000). In developed countries daily requirement of vitamin E could be easily fulfilled but in the developing countries vitamin E deficiency is common in premature infants

and elderly people (Sokol, 1988). Maize grain is a major component in diets of humans and animals and important sources of vitamin E. Total tocopherol, consisting α-, β-, γ- and δ-isoforms, are mainly concentrated in the germ (Weber, 1987). However, γ-tocopherol predominates with ~80% while α -tocopherol constitutes only ~20% of the total tocopherol content (Galliher *et al.* 1985). Alfa tocopherol is a more potent anti-oxidant than γ-tocopherol so increased of α-tocopherol content in maize seeds would help in enhancing the vitamin E activity in maize. There is substantial variation for kernel levels of tocopherols (Chander *et al.* 2008; Li *et al.* 2012; Lipka *et al.* 2013) that can be exploited to improve the vitamin E content through biofortification.
The aim of this study is to investigate of the tocopherols variation in different maize germplasm and to identify of genotypes with high content of γ- and α-tocopherols.

Material and methods

One hundred twenty three maize inbred lines, 74 with standard kernel type (1-74), 37 sweet corn (75-111) and 12 popcorn lines (113-123) were analyzed for tocopherols content by high performance liquid chromatography (HPLC). Maize grains were milled (Perten 120, Sweden) into flour (particle size less than 500μm) and stored at -20˚C prior to analysis. Standards for tocopherol (α-, γ+β and γ) were produced by Sigma Aldrich (Germany). Maize sample (0.2 g) was mixed with 2-propanol (4 mL) and homogenized for 30 min at room temperature (Gliszczyńska-Świgło *et al.*, 2007). The extracts were then centrifuged at 3000 rpm for 5 min, filtered through a 0.45 μm membrane filter and the clear supernatant was directly injected in the same liquid chromatography system equipped with fluorescence detector (FLD-3100) and the same analytical column maintained at 30˚C. Mixture of acetonitrile and methanol (1:1, v/v) was used as a mobile phase (1 mL/min), injection volume was 5μL, and the wavelengths for excitation and emission were set at 290 nm and 325 nm, respectively. Tocopherols were identified and quantified comparing characteristic retention time of corresponding standards. The tocopherol content is expressed as μg/g DW.

Results and discussion

The study revealed wide genetic variation for the kernel tocopherols in maize inbred lines with different kernel type. The mean values of δ, β + γ and α-tocopherol were 1.89, 61.41 and 12.27 μg/g DW, respectively. The highest mean value of δ-tocopherols 2.11 μg/g DW have was recorded in sweet corn inbred lines followed by inbred line with standard kernel type (1.82 μg/g DW) and lower values were fount in one popcorn inbred lines (1.36 μg/g DW). Sweet corn inbred lines have the highest mean value of ß+γ-tocoherols (72.81 μg/g DW) and whereas, the lowest values were recorded in one popcorn inbred lines (43.16 μg/g DW). The highest α-tocopherol content was recorded in inbred lines with standard kernel type followed by sweet corn inbred lines and popcorn lines (14.72, 9.93 and 4.23μg/g DW respectively).
In the reverse phase system, which was employed in this study, there is no possibility to separate β- and γ- tocopherol, so their content is expressed as their sum (Gliszczyńska-Świgło *et al.*, 2004, Abidi, 2009). The genetic variation for various tocopherols among maize inbred lines showed that β+γ tocpherols were the most predominant tocopherols as was previously shown by Combs and Combs (1985). The β+γ tocopherol content varied from 30.79 to 105.62 μg/g DW in inbred lines with standard kernel type, 42.56-115.65 μg/g DW in sweet corn inbred lines and 32.91 to 62.45 μg/g DW in popcorn inbred lines. Sweet corn inbred lines have the highest average value of β+γ tocopherols (72.81 μg/g) followed by standard kernel inbred lines (58.82 μg/g), and popcorn (43.16 μg/g).

Table1. Content of tocopherols in 123 maize inbred lines and α/γ ratio

No	δ-tocopherol	ß+γ tocopherol	α-tocopherol	α/γ	No	δ-tocopherol	ß+γ tocopherol	α-tocopherol	α/γ
1	1.32±0.01	30.79±0.13	9.72±0.05	0.32	63	1.42±0.02	55.12±0,30	14.68±0.08	0,38
2	1.31±0.01	40.21±0.30	10.00±0.05	0.25	64	1.06±0.02	59.18±0,25	23.18±0.20	0,39
3	1.29±0.01	80.21±0.22	24.28±0.05	0.30	65	0.96±0.08	80.83±0,98	3.41±0.04	0,04
4	1.59±0.05	80.10±0.56	9.72±0.03	0.12	66	3.41±0.01	60.23±0.19	24.48±0.08	0,41
5	1.44±0.03	80.16±0.39	11.56±0.04	0.14	67	0.81±0.00	53.33±0.10	19.12±0.08	0.36
6	2.43±0.06	105.62±1.37	6.94±0.08	0.06	68	0.59±0.00	51.21±0.18	20.52±0.17	0.40
7	1.94±0.04	92.89±0.88	9.25±0.06	0.09	69	1.60±0.04	65.61±0.55	14.88±.0.12	0.23
8	2.18±0.05	99.25±1.12	8.10±0.07	0.08	70	1.41±0.03	68.95±0.78	13.26±0.26	0.19
9	1.05±0.02	61.78±0.19	8.22±0.01	0.13	71	0.75±0.04	70.52±0.66	5.27±0.05	0.07
10	0.72±0.01	33.07±0.17	15.41±0.21	0.46	72	0.93±0.06	70.36±1.00	8.92±0.07	0.13
11	0.88±0.01	47.43±0.18	11.81±0.11	0.25	73	2.02±0.01	61.88±0.27	19.78±0.13	0.32
12	3.08±0.02	68.90±0.68	5.72±0.05	0.08	74	1.20±0.01	71.21±0.20	20.24±0.13	0.28
13	0.88±0.02	62.24±0.44	7.84±0.03	0.12	75	1.56±0.03	70.36±0,45	8.50±0.02	0.12
14	1.45±0.04	72.13±0.66	6.72±0..03	0.09	76	2.23±0.04	43.50±0.86	6.65±0.13	0.16
15	1.22±0.01	33.89±0.16	13.35±0.06	0.39	77	1.36±0.01	75.05±0.19	9.63±0.01	0.13
16	0.61±0.00	35.74±0.13	18.70±0.07	0.52	78	1.51±0.03	72.28±0.56	5.65±0.04	0.08
17	1.20±0.00	43.97±0.11	12.37±0.03	0.28	79	1.24±0.06	57.47±1.04	9.31±0.07	0.16
18	1.41±0.02	52.81±0.36	10.78±0.05	0.20	80	0.93±0.03	11.65±0.66	5.19±0.03	0.04
19	1.13±0.01	50.13±0.31	11.63±0.04	0.23	81	1.91±0.03	88.55±0.72	8.29±0.07	0.09
20	1.17±0.01	80.10±0.56	9.72±0.03	0.22	82	2.68±0.02	55.53±0.31	5.53±0.03	0,10
21	1.15±0.01	50.80±0.32	11.42±0.04	0.23	83	2.72±0.07	56.96±1.44	11.64±0.30	0.20
22	1.16±0.01	51.13±0.33	11.31±0.04	0.22	84	3.64±0.01	71.64±0.15	12.29±0.03	0.17
23	1.15±0.01	50.97±0.32	10.36±0.04	0.20	85	4.10±0.08	85.15±1.66	16.06±0.31	0.19
24	1.16±0.01	101.85±0.33	10.83±0.04	0.11	86	1.03±0.03	74.68±0.60	8.69±0.08	0.12
25	4.27±0.03	71.70±0.53	21.73±0.16	0.30	87	2.56±0.06	79.92±1.13	12.38±0.20	0.16
26	2.71±0.02	86.78±0,43	16.28±0.10	0.19	88	1.80±0.04	77.30±0.87	10.53±0.14	0.14
27	3.49±0.03	79.24±0.48	19.01±0.13	0.24	89	0.61±0.03	109.21±0.59	12.52±0.08	0.12
28	3.10±0.02	83.01±0.45	17.64±0.12	0.21	90	2.31±0.06	95.38±1.12	14.31±0.16	0.15
29	3.42±0.04	42.20±0.69	16.28±0.44	0.38	91	0.71±0.03	82.49±0.68	10.91±0.08	0.13
30	0.21±0.03	56.27±0.61	15.79±0.15	0.28	92	1.21±0.05	81.81±0.93	10.61±0.04	0.12
31	1.82±0.04	49.24±0.65	16.04±0.29	0.32	93	1.14±0.02	47.23±0.40	11.60±0.10	0.25
32	1.11±0.02	56.16±0.56	8.10±0.07	0.14	94	1.80±0,01	53.88±0,21	13.73±0.05	0.26
33	3.39±0,02	70.69±0.40	20.21±0.12	0.28	95	3,66±0,02	75.26±0,34	10.68±0,05	0.14
34	1.82±0,01	38.89±0.31	18.52±0.14	0.48	96	2,31±0,02	60.48±0,50	14.11±0.12	0.24
35	2.61±0.06	54.79±0.91	19.37±0.09	0.35	97	2,12±0,03	53,21±0,78	6.31±0.09	0,12
36	2.21±0.05	46.84±1.04	18.94±0.08	0.40	98	2,91±0,05	81,21±1,43	8,31±0.14	0,10
37	2.41±0.06	50.82±0,97	19.15±0.08	0.38	99	2,21±0,02	83,87±0,30	9,01±0.03	0,11
38	2.31±0.06	48.83±1,01	19.05±0.09	0.39	100	2,21±0,02	63,21±0,38	8.31±0.05	0,13
39	1.21±0,01	31.19±0,34	21,83±0.23	0.69	101	2,18±0,02	55.62±0.59	10.99±0.02	0,19
40	0.47±0.03	55.20±0.21	18.73±0.27	0.33	102	3 20±0, 3	69 46±0.63	6.94±0.06	0 09
41	2.79±0.01	55.12±0.30	14.68±0.08	0.27	103	1.36±0.04	42.56±0.55	4.17±0.05	0 10
42	3.10±0.01	52.29±0.23	11.39±0.06	0.22	104	3.64±0.06	65.01±1.00	12.12±0 19	0 19
43	2.48±0.02	57.94±0.37	17.96±0.11	0.31	105	1 46±0.06	84.38±0.92	7.30±0.08	0 08
44	1.62±0.02	53.21±0.78	15.81±0.21	0.29	106	2 64±0.01	53.90±0.23	6 71±0.03	0 13
45	2.05±0.02	55.58±0.57	16.89±0.16	0.30	107	1 35±0 05	92.41±0.78	6 26±0.05	0 07
46	1.26±0.01	78.21±0.20	17.21±0.20	0.22	108	4 01±0.02	103.21±0.54	19.01±0.09	0.18
47	1.06±0.04	41.91±0.13	17.75±0.05	0.42	109	2. 21±0.28	59.99±0.28	13.98±1.36	0.23
48	3.21±0.05	68.72±1.03	16.22±0.24	0.24	110	2 21±0 02	63.21±0.38	8.31±0.05	0.13
49	1.20±1.02	52.12±0.13	15.21±0.14	0.29	111	1 29±0 05	64.47±0.95	10,70±0.16	0.16
50	1.22±0.02	32.02±0.30	13.51±0.21	0.42	112	2.31±0,02	36,33±0,37	6.36±0.06	0.17
51	1.38±0.01	36.31±0.08	8.60±0.01	0.23	113	1.45±0,06	40.27±0.30	5.20±0.01	0.13
52	1.67±0.03	45.05±0.82	10.05±0.09	0.22	114	1.73±0.07	39.19±0.56	4.14±0.02	0.10
53	3.74±0.04	92.71±0.77	18.43±0.15	0.19	115	2.03±0,02	38.08±0.16	5.60±0.02	0.15
54	3.10±0.05	81.10±1.23	23.42±0.36	0.29	116	0.98±0.01	35.15±0.12	3.50±0.01	0.09
55	2.01±0.03	51.91±0.89	18.90±0.31	0.37	117	0.70±0.04	39.73±0.39	3.12±0.01	0.08
56	2.88±0.02	52.56±0.40	15.27±0.11	0.29	118	1.21±0.02	32.91±0.21	4.01±0.02	0.12
57	2.81±0.02	43.21±0.33	7.54±0.06	0.18	119	1.51±0.05	50.22±0.44	5 86±0.03	0.12
58	1.52±0.03	41.24±0.67	15.21±0.25	0.37	120	1.21±0.01	48.11±0.17	4.53±0.01	0.09
59	3.81±0.03	82.29±0.49	16.96±0.10	0.21	121	0.81±0.03	43.21±0.39	3.75±0.02	0.09
60	1.18±0.01	52.34±0.20	16.66±0.11	0.32	122	1.21±0.02	62.45±0.19	4.49±0.01	0.08
61	3.31±0.03	81.76±0.46	11.21±0.06	0.14	1.23	1.22±0.22	52.23±0.20	4.21±0.01	0.08
62	1.89±0.02	63.22±0.42	25.47±0.32	0.40					

The above results are in accordance with the respective of our previous study of tocopherols content in different set of inbred lines (Drinic *et al*. 2019) where sweet corn inbred lines had higher $\beta+\gamma$ tocopherols content than standard kernel type inbred lines. Ibrahim and Juvik (2009) studied 41 sweet corn genotypes and found that the most abundant form of tocopherols was γ -tocopherol.

Significant variability was detected for δ-tocopherols and α-tocopherol content, e.g. from 0.21 to 4.27μg/g DW and 3.12 to 25.47 μg/g DW, respectively (Table 1). Average values of α tocopherol in standard kernel type, sweet corn and popcorn inbred lines were 14.72, 9.93 and 4.23μg/g DW, respectively. The content of α-tocopherol varied from 3.41 to 25.47μg/g DW in standard kernel type inbred lines, followed by sweet corn (4.17-19.01 μg/g DW) and popcorn (3.12-6.36 μg/g DW). Several studies have shown significant differences among maize inbreds for tocopherol levels. Similar range of variation (3.4 to 34.3 μg g−1) for kernel α-tocopherol in maize has been reported by Muzhingi *et al.* (2017). A much higher range of variation for α-tocopherol (0.40 to 61.08 μg g−1) was reported by Li *et al.* (2012). The content and composition of tocopherols may vary among different maize varieties owing to genotypic variation and difference in geographical origin, agricultural factors, and analytical methods (Panfili *et al.* 2003; Chander *et al.*, 2008; Ali *et al.* 2010).

The highest level of α tocopherol was recorded in ~~have~~ inbred line TVA973 (25.47μg/g) followed by inbred line H (24.28 μg/g) and lowest one 3.12 μg/g popcorn inbred line ZP613 (3.12 μg/g). The γ tocopherol content was highest in sweet corn Gold 5 (115.62 μg/g), followed by the Esteem-F1-6 (109.21 μg/g), and was lowest in A417 (30.79 μg/g). The distribution of different tocopherols in the inbred lines also varied. In F135 γ-toocpherols content (33.07 μg/g DW) was nearly twice higher that of α-tocpherols (15.41μg/g). even Similarly, in sweet corn inbred lines Gold 5 ß+γ content (115,25μg/g) was more than twenty times as that of α-tocopherols (5,19 μg/g). (Table 1). Higher amount of α-tocopherol and equitable ß+γ with standard kernel type resulted into higher proportion of α-/ ß+γ tocopherols (0.27) compared to the respective value (0.11) in popcorn (0.11).

Most maize breeding lines naturally have much more γ-tocopherol than α-tocopherol. Kurilich and Juvik (1999) found that γ-tocopherol was predominant among 44 maize varieties, with most at and in two-thirds of total tocopherol, α-tocopherol contributed up to 27%, and δ-tocopherol to only 4%. The level of most important, α-tocopherol is about 20% of total tocopherols in maize (Rocheford *et al*, 2002), and its higher levels can be obtained through either utilization of existing natural variation or by genetic engineering. Wide variation for α-, and β+γ- tocopherol among 123 diverse maize inbreds revealed a scope for genetic improvement. α-tocopherol because of its higher biological activity is more desirable for human and animal consumption than γ-tocopherol. However, recent research suggests that γ-tocopherol and compounds metabolized from it have properties important to human health that are unique compared to properties of α-tocopherol. Therefore it may be desirable to not only increase levels of α-tocopherol, but also levels of γ-tocopherol.

Conclusions

Maize lines differ greatly in their composition and total seed content of tocopherols. Standard kernel type inbred lines had the highest value of α –tocopherol, whereas sweet corn inbreds had the highest value of ß+γ tocopherols. Demonstrated natural variations of tocopherols content in maize suggest a genetic potential for breeding tocopherols improved maize. Tocopherols are compounds present in maize that provide health and economic benefits, which potentially could be captured by both producers and consumers to add value to the grain.

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ANALYSIS OF VEGETABLE PROTEINS BY CAPILLARY GEL ELECTROPHORESIS (CGE)

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Abstract

Protein content in plant species varies depending on the species and stage of maturity. In plants, proteins represent a structural material and show characteristic activity and function. Therefore, proteins are carriers of basic life functions in the cell. Different techniques can be used to separate and identify proteins. The aim of this study was to examine the possibilities of applying SDS-capillary gel electrophoresis (SDS-CGE) for separation and quantitative analysis of vegetable proteins. Potato and carrot samples were used as test samples. Extraction of total proteins was performed, and separation of proteins from vegetables was carried out by capillary gel electrophoresis (CGE), using the SDS-MW Analysis Kit (Beckman Coulter). During protein analysis by capillary gel electrophoresis, a mixture of 7 proteins of known molecular weights (10 kDa, 20 kDa, 35 kDa, 50 kDa, 100 kDa, 150 kDa and 225 kDa) was used as the Mw standard. On the electrophoregram, obtained after the analysis of potato proteins, 31 proteins of different molecular weights were separated. After the analysis of carrot proteins, 21 proteins of different molecular weights were separated. Proteins with molecular weights of 50-100 kDa had the highest relative protein concentrations, in the amount of 44.94% in potato and 53.03% in carrot. This method enables the identification and quantification of vegetable proteins and it can be used to study the changes and behavior of proteins in different growing and processing conditions of vegetables.

Keywords: *Proteins, Vegetables, Capillary Gel Electrophoresis.*

Introduction

In all living systems, including plants, proteins play two essential and different roles: they represent structural material and show specific activity and function, which makes them the carriers of basic life functions within a cell. All life processes of a cell take place in or with protein structures. Proteins are catalysts of numerous biochemical processes within a cell (Popović, 2005). There are several thousands of proteins in plant cells (Wadhave et al., 2014). Plant proteins may be divided into two basic groups. The first group are storage (reserve) proteins that do not have functional activities, but serve as a depot of amino acids which, if necessary, are used for plant growth and development. The second group is represented by functional proteins: enzymes, hormones, protective proteins and structural proteins (Devappa et al., 2010). All plants contain proteins. The protein content varies depending on the type and stage of maturity, with the protein content decreasing during the maturation of the plant. Protein fractions can change during storage and processing of plant material (Wadhave et al., 2014). Plant proteins differ from each other in the number and order of amino acid residues, polypeptide chains, molecular weight, etc. The biological function of proteins is closely related to their physicochemical properties and hence those properties are being intensively studied (Popović, 2005; Bonczar et al., 2016; Sharma et al., 2018; Rasheed et al., 2020).

Different methods can be used to separate and identify proteins. Protein separation using Capillary Gel Electrophoresis (CGE) is based on the difference in electrophoretic mobility of ions in the medium in narrow capillaries. The molecule size affects ion mobility (Sirén, 2015). When the sample is treated in a buffer containing sodium dodecyl sulfate (SDS), the technique is known as capillary-based SDS-CGE. This method allows rapid separation of peptides and proteins from complex systems such as various food products (Vallejo-Cordoba et al., 2010; Stepanova and Kasicka, 2016; Bonczar et al., 2016).
The aim of this paper was to examine the possibilities of applying SDS-capillary gel electrophoresis (SDS-CGE) for separation and quantitative analysis of vegetable proteins from potato and carrot samples.

Material and Methods

Potato and carrot samples were used as test samples. Fresh samples of potatoes and carrots were brought from a local supermarket. The analysis was performed immediately after the purchase of samples, at the Faculty of Technology Zvornik, University of East Sarajevo. Total proteins were extracted from the vegetables. The vegetable samples (45 mg) were suspended in equal volumes of treatment buffer: 0.125 M tris-Cl pH 6.8, 4% sodium dodecyl sulphate, 20% glycerol, 10% 2-mercaptoethanol and deionized water and then intensively homogenized 10 using Vortex (Velp, Italy). The resulting extract was clarified by centrifugation at 14,000 rpm for 20 min and the supernatant containing total proteins was decanted. Due to the low protein concentration, the protein extracts were evaporated in a Reacti-Therm I module (Thermo Fisher Scientific Bellefonte, PA., USA) to a dry residue at room temperature.
Protein separation was performed using capillary electrophoresis (Agilent, CE 7100) and the SDS-MW Analysis Kit (Beckman Coulter). The content of the SDS-MW Analysis Kit is: 2 capillaries 57 cm long, 50 μm I.D; SDS-MW Gel buffer – appropriate formulations; SDS Sample buffer – 100 mM Tris-HCl pH 9.0 / 1% SDS; SDS Protein Standard (10 to 225 kDa); Internal standard 10 kDa; 0.1 N HCl; 0.1 N NaOH. To prepare the SDS Mw standard, 10 μL of the Mw Standard needs to be mixed with 85 μL of Sample buffer, 2 μL of internal standard and 5 μL of 2-mercaptoethanol. After gentle stirring, it was heated to 100 °C (Thermo - Shaker, Biosan, TS100) for three minutes and the content was cooled to room temperature before injection. Samples of extracted proteins from the tested vegetable samples were dissolved in SDS buffer to a total volume of 100 μL, and then filtered through 0.2 μm pore size filters. After that, 2 μL of internal standard was added. The contents were gently stirred and then heated for 3 minutes at 100 °C (Thermo - Shaker, Biosan, TS100) to ensure denaturation of the proteins present and cooled to room temperature before injection. During protein analysis by capillary gel electrophoresis, injection of the prepared Mw standard was performed first, with subsequent injection of the prepared samples. Separation of proteins was performed in a capillary with an inner diameter of 50 μm, a total length of 33 cm, and an effective length of 23.50 cm. SDS gel buffer of appropriate composition was used to fill the capillary. The detection wavelength was 220 nm with 20 nm permeability (without reference wavelength), and a response time was 1 second. All preparation procedures of the system were performed in accordance with the recommendations of the system manufacturer (Wenz, 2011). Once a day, the capillary was conditioned using the following procedures: washing under a pressure of 2 bar with 0.1 N NaOH for 10 minutes, then 0.1 N HCl for 5 minutes and ultrapure water for 2 minutes. Prior to each injection, the capillary was flushed for 3 minutes with 0.1 N NaOH at a pressure of 4 bars, then 0.1 N HCl at a pressure of 2 bars for 1 minute and with ultrapure water at a pressure of 2 bars for 1 minute. The capillary was filled with SDS-gel buffer under a pressure of 4 bar for 10 minutes, after which the input electrodes were briefly immersed in water (for 2 seconds). The injection was performed electrokinetically

using a voltage of -5 kV for 20 seconds, after which the input electrodes were briefly immersed in water (2s). Separation is performed for 30 minutes, using a voltage of -16.5 kV. Upon completion of the analysis, the capillary was flushed for 15 minutes with 0.1 N NaOH at a pressure of 4 bars, then 0.1 N HCl at a pressure of 3.5 bars for 5 minute and with ultrapure water at a pressure of 3.5 bars for 10 minute. 2-ml glass vials were used for all reagents. Reagent filling volume was 1.20 ml, except for vials filled with 1.6 mL water and, while 3 vials for reagent residues (0.1 N NaOH, 0.1 N HCl / water and SDS gel buffer) contained 0.6 ml of water.

Figure 1 shows the electropherogram of the known molecular weights (Mw) standard. The standard contains seven proteins of known molecular weights (10kDa, 20kDa, 35kDa, 50kDa, 100kDa, 150kDa and 225kDa). Based on migration times and known molecular weights, a calibration curve was obtained (Figure 2) which was used to estimate the molecular weights of unknown proteins. Qualitative and quantitative analyses of proteins involve determining the molecular weights of separated proteins and concentration of separated proteins respectively. The relative protein concentration was determined using ChemStation Software (Agilent). Quantification of the relative concentration of each protein in the mixture was performed based on the area of peaks on the obtained electropherograms.

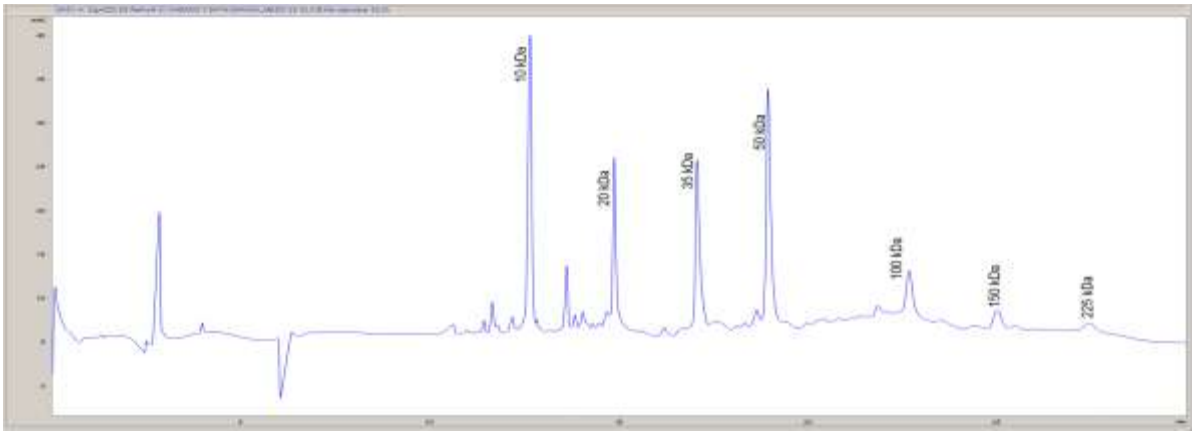


Figure 1. Electropherogram of proteins of known molecular masses (Mw standard)

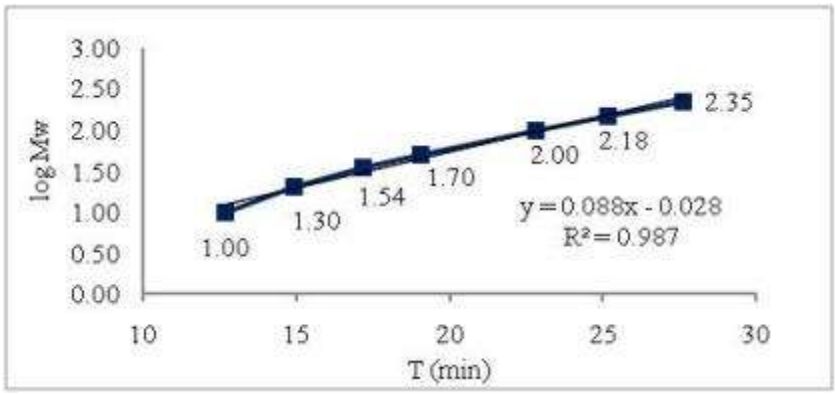


Figure 2. Calibration curve for estimating molecular weights of unknown proteins

Results and Discussion

Figure 3 shows the electropherograms obtained after separation of proteins from the potato and carrot samples. The results obtained by capillary gel electrophoresis showed that the molecular weights of proteins isolated from fresh potatoes and carrots range from 0 to 100 kDa. Table 1 shows the number and relative concentration of total proteins obtained from the

tested samples using separation by CGE. On the electrophoregram obtained after the analysis of potato proteins, 31 proteins of different molecular masses were separated. The largest number of protein units was recorded in the weight range from 0 kDa to 20 kDa (24 proteins), while the number of proteins of higher molecular weights was significantly lower – five proteins weighing 20-50 kDa and two proteins weighing 50-100 kDa. The relative concentration of 0-20 kDa potato protein was 41.89%. Proteins weighing 20-50 kDa were present in the amount of 13.17%, while proteins weighing 50-100 kDa had the highest relative concentration of 44.95%. Vegetables contain relatively small amount of proteins. The total protein content in potatoes is about 2% of the total weight or approx. 10% of the dry matter. However, the protein content of potatoes can vary significantly depending on the genotype and growing conditions (Bartova et al., 2009). Due to the favourable amino acid composition and good digestibility, potato proteins are suitable for human consumption (Bartova and Barta, 2008). The soluble proteins present in potato juice (after removing starch) can be divided into three groups. The first group of proteins are patatin proteins, which make up 30-40% of the total amount of proteins present in potatoes. The second group consists of protease inhibitors, which are present from 40 to 50%. The third group of potato proteins are other proteins, mainly enzymes such as kinases and enzymes involved in starch synthesis, with 10 – 15% presence (Alting et al., 2011). Patatin has a high nutritional value and is the most important storage protein of potatoes. The content of patatin in potatoes significantly depends on the potato variety and climatic conditions (Bartova and Barta, 2008). The molecular mass of patatin is 40-42 kDa, and the isoelectric point is between 4.5 and 5.2. At neutral pH and standard temperature, patatin exists as a dimer whose structural units are linked by noncovalent hydrophobic bonds. Many soluble and some insoluble proteins are composed of two or more polypeptide chains or subunits (dimer, trimer or tetramer) that may be the same or different. These subunits can be isolated from concentrated saline or under mild denaturing conditions (Popović, 2005). Racusen and Weller (2007) reported that patatin, the major potato protein, exists as a dimer of 88 ± 4 kDa in dilute buffers at pH 7 or 9.6. The dimer was found in fresh as well as lyophilized preparations. Patatin has a high degree of secondary structure, low denaturation temperature (60 °C at pH 7.0) and relatively low stability as a function of pH (loss of structure at pH ≤ 4.5). Unlike other storage proteins, patatin possesses certain enzymatic activities, which is crucial for its functional properties (Anderson et al., 2002; Alting et al., 2011). Protease inhibitors (PIs) are a heterogeneous group of proteins, which are the most abundant proteins in potatoes. Bartova and Barta (2008) state that the molecular mass of these proteins ranges from 4.3 to 24 kDa, and that these proteins are more thermally stable than patatin, although temperatures above 45 °C cause denaturation and insolubility of most of these proteins. Other potato proteins do not belong to the patatin group or do not show protease inhibitor activity. They have a molecular weight greater than 40 kDa and include lectins, polyphenol oxidase, lipoxygenase, enzymes involved in starch synthesis, and phosphorylase isoenzymes (Alting et al., 2011). On the electrophoregram obtained after the analysis of carrot proteins, 21 proteins of different molecular masses were separated (Figure 3). It was determined that, out of the total number of separated proteins, 19 proteins had a molecular mass of 0-20 kDa and two proteins had a molecular mass of 50-100 kDa. The relative concentration of carrot protein mass 0-20 kDa was 46.97%, and proteins with the mass 50-100 kDa were present at 53.03% concentration (Table 1). Proteins are biological macromolecules responsible for most biological functions within the cell. Plants contain a number of specific types of proteins that are not found in other living organisms. Like proteins in general, plant proteins have different enzymatic, structural, and functional roles (photosynthesis, biosynthesis, transport, immunity, etc.). They also act as storage mediums to meet the growth and nutritional demands of developing seedlings (Rasheed et al., 2020). Carrot root has been reported to contain an anti-freeze protein, which has a molecular weight

of 36.8 kDa. Carrot anti-freeze protein has a strong anti-recrystallization ability and other cryoprotective properties (Sharma et al., 2018). Plant proteins are sensitive to environmental and processing conditions. Denaturation of plant proteins can occur during extraction, food processing or storage, which can potentially affect their properties and functional properties in food systems (e.g. in emulsions and foams) (Lin et al., 2017).

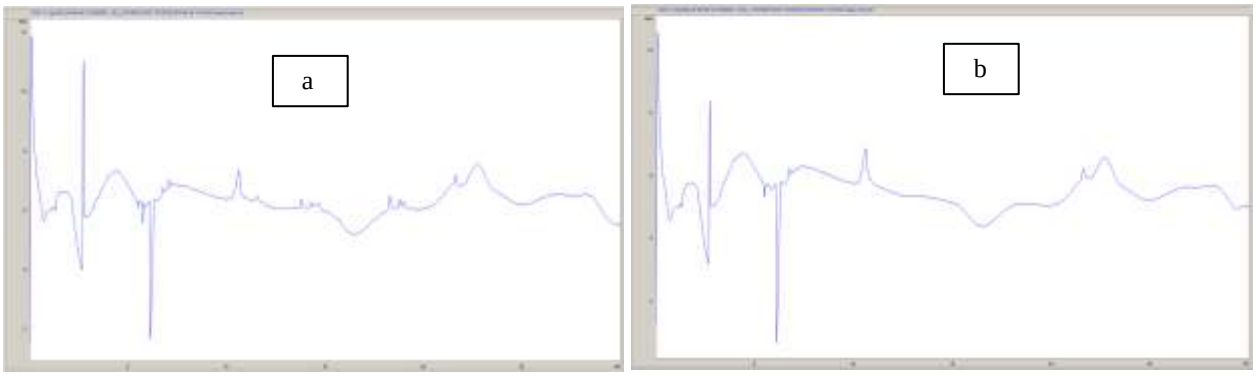


Figure 3. Electrophoregrams obtained after electrophoretic separation of potato (a) and carrot (b) proteins.

Table 1. Total number of proteins and relative concentration of proteins obtained during separation by CGE from vegetable samples

Molecular mass (kDa)	Potato		Carrot	
	Number of proteins	Relative concentration (%)	Number of proteins	Relative concentration (%)
0-20	24	41.89	19	46.97
20-50	5	13.17	0	0
50-100	2	44.94	2	53.03
100-150	0	0.00	0	0
>150	0	0.00	0	0

Conclusions

Electrophoretic separation of proteins by capillary gel electrophoresis (CGE) is simple, fast and efficient. If appropriate software is used, after separating the proteins in the capillary and determining the migration time, CGE allows the calculation of molecular masses and relative protein concentrations from the tested samples. Capillary gel electrophoresis (CGE) enables the separation of plant proteins and the determination of the molecular mass of separated proteins in the range from 10 kDa to 225 kDa. After analysis of the potato proteins by capillary gel electrophoresis, 31 proteins of different molecular weights were isolated. Twenty-one proteins of different molecular weights were separated by the carrot proteins analysis. Proteins with molecular mass of 50-100 kDa had the highest relative concentrations of proteins (44.94% in potatoes and 53.03% in carrots). This method can be used to study changes and behaviours of proteins in different conditions of vegetable growing and processing.

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INFLUENCE OF COAGULATION CONDITIONS ON THE CONTENT OF FATTY ACIDS IN ACID-COAGULATED CHEESES

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Abstract

Cheese is a product formed by the separation of whey after coagulation of milk. Milk coagulation can be done in several ways. Acid-coagulated cheese is produced by the action of high temperatures with the addition of organic acids. The aim of this study was to examine the influence of coagulation temperature and coagulant type on the content of saturated and unsaturated fatty acids in cheese, obtained by heat-acid coagulation of milk. Protein coagulation was performed at a temperature of 85°C (samples 1, 3, 5) and 95°C (samples 2, 4, 6), and three organic acids were used as coagulants, namely: citric acid (samples 1 and 2), tartaric acid (samples 3 and 4) and acetic acid (samples 5 and 6). Determination of the qualitative and quantitative composition of fatty acids in the tested samples was performed by GC analytical technique. Palmitic (C16:0), stearic (C18:0) and myristic (C14:0) acids were the most abundant of the saturated fatty acids in the tested cheese, and oleic acids (C18:1) of the unsaturated fatty acids. The palmitic acid content ranged from 30.61% (sample 3) to 31.00% (sample 5) of the total amount of identified fatty acids. The stearic acid content ranged from 11.57% (sample 3) to 11.79% (sample 5), while the myristic acid content ranged from 10.89% (sample 3) to 10.98% (samples 5 and 6) of the total fatty acids identified. The oleic acid content ranged from 24.05% (sample 3) to 24.40% (sample 6) of the total amount of identified fatty acids. Other identified fatty acids were present in smaller amounts in the tested samples.

Keywords: *Milk, Cheese, Fatty Acids.*

Introduction

Milk fat is a very important ingredient in milk and plays a significant role in cheese production. It adds to the quality and yields a larger amount of curd giving a special flavour and aroma to the products (Singh and Cadwallader, 2008; Parodi, 2009). Milk fat is composed of a large number of fatty acids. It has been discovered that milk contains more than 400 different fatty acids. A large number of fatty acids make the biological value of butterfat great because the organism can pick the necessary ones but cannot synthesize them (Parodi, 2009). Fatty acids vary in chain length (2 to 20 carbon atoms) and saturation (0 to 4 double bonds) (Walstra et al., 2006). Fatty acids in milk include saturated and branched chain fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids (Kailasapathy, 2008).

A typical milk fat consists of 62% saturated, 29% monounsaturated and 4% polyunsaturated fatty acids. At room temperature, saturated fatty acids are solid, while unsaturated fatty acids are liquid. Dietary fats that are rich in saturated fatty acids involve an increased risk of cardiovascular diseases. In contrast, fats rich in cis-monounsaturated fatty acids and n-3 long-chain polyunsaturated fatty acids have been proven to play a useful role in heart disease prevention (Chandan et al., 2008). Unsaturated fatty acids with multiple double bonds are functional, healthy and necessary for the human body (Kilara and Chandan, 2008). Fatty acids

can be altered by physical and chemical processes during the processing and storage of milk and dairy products, such as oxidation and formation of trans-fatty acids, etc. (Herzallah et al., 2005). Large quantities of milk are processed to produce various kinds of cheese, which represent the most important and most numerous group of dairy products. Cheese is obtained by coagulation of milk along with separation of whey. Various organic acids can be used as coagulants in the production of cheese, like citric or lactic acid, calcium lactate, lemon juice, sour whey, etc. (Khan et al., 2014; Kumar et al., 2015; Cao et al., 2017; Bankar et al., 2018). Depending on the coagulants used, various kinds of cheese with different physical, chemical and sensory properties are obtained (Zhang et al., 2013; Oštarić et al., 2015; Shanaziya et al., 2018). Acid-coagulated cheeses are formed by lowering the pH value to reach the isoelectric point of casein, by lactic acid fermentation, or by adding different types of organic acids to milk. A specific group of acid-coagulated cheeses consists of those obtained by heat-acid coagulation i.e. the activity of organic acids at high temperatures (Jovanović et al., 2000; Popović-Vranješ, 2015; Chinprahast et al., 2015).

The aim of this study was to examine the influence of coagulation temperature and type of coagulant on the content of saturated and unsaturated fatty acids in cheeses obtained by heat-acid coagulation of milk.

Material and Methods

Pasteurized cow's milk containing 3.2% fat (produced by Pađeni Dairy, Bileća from the Entity of Republic of Srpska, Bosnia and Herzegovina) bought in a local supermarket was used to produce cheese. The milk was stored in the refrigerator at a temperature of + 4 °C before it was processed into cheese. Cheese production was carried out in the milk processing plant (mini cheese factory) at the Agricultural School, Banja Luka. Six models of cheese samples were produced for this experiment (Table 1). Fresh pasteurized milk was heated to the appropriate temperature (85 °C and 95°C), and various organic acids (citric, tartaric and acetic acid) were used to coagulate the milk. After the addition of acid, the mixture was mixed well and rested for 10 min to obtain curd. The resulting curd was filtered through gauze into a strainer. The hot drained curd was weighed and 1.5% of salt was added in it. After salting and mixing, the curd was placed for pressing in a mould. The pressing took one hour, and the load was 2 kg per one kilo of cheese. The finished cheese was cooled in a refrigerator to +4 °C and stored at the same temperature until the analysis.

Table 1. Acid-coagulated cheese production requirements

Sample	Type of coagulant	Amount of coagulant (%)	Coagulation temperature (°C)
1	Citric acid	0.30	85
2	Citric acid	0.30	95
3	Tartaric acid	0.30	85
4	Tartaric acid	0.30	95
5	Acetic acid (9%)	1.50	85
6	Acetic acid (9%)	1.50	95

The analysis was performed 24 hours after production, at the Faculty of Technology, University of Banja Luka. Fatty acid methyl esters of the tested samples were prepared by direct esterification with saturated KOH/methanol solution. The equipment used to determine fatty acids was Clarus 680 Perkin Elmer gas chromatograph with FID detector, Elite-wax L 60 m column, ID 0.32, DF 0.5, with the standard: Sigma Aldrich Supelco 37 components FAME MIX. Injector and detector temperature 250 ºC, sample volume 1µL, temperature regime 60 ºC for 2 minutes, 10ºC/min up to 200 ºC, 5 ºC/min up to 240 ºC and 30 min at 240.

The total was duration 54 min, flow 1.5 mL / min, nitrogen gas (carrier), hydrogen and air (for FID). The composition of fatty acids is shown as a percentage of individual fatty acids in the total amount of identified fatty acids (g/100 g of total fatty acids).
Statistical processing of the obtained results was performed using the Microsoft Excel 2013 software package and the IBM SPSS Statistics 22.0 computer program for Windows (Armonk, NY, United States). The results obtained in this paper are presented as mean values of the individual results of three randomly selected product samples ± standard deviation (SD). The significance of differences between arithmetic means was determined by analysing the variance with one independent variable and multiple interval tests and expressed with 95% probability.

Results and Discussion

Based on the obtained results, it was established that the content of saturated acids in the examined cheeses was much higher than the content of unsaturated fatty acids (Figure 1). The total content of saturated fatty acids ranged from 65.62 (sample 3) to 66.12% (sample 1). The content of monounsaturated fatty acids ranged from 27.15 (sample 3) to 27.94% (sample 6), and the content of polyunsaturated fatty acids ranged from 6.22% (sample 6) to 7.21% (sample 3).

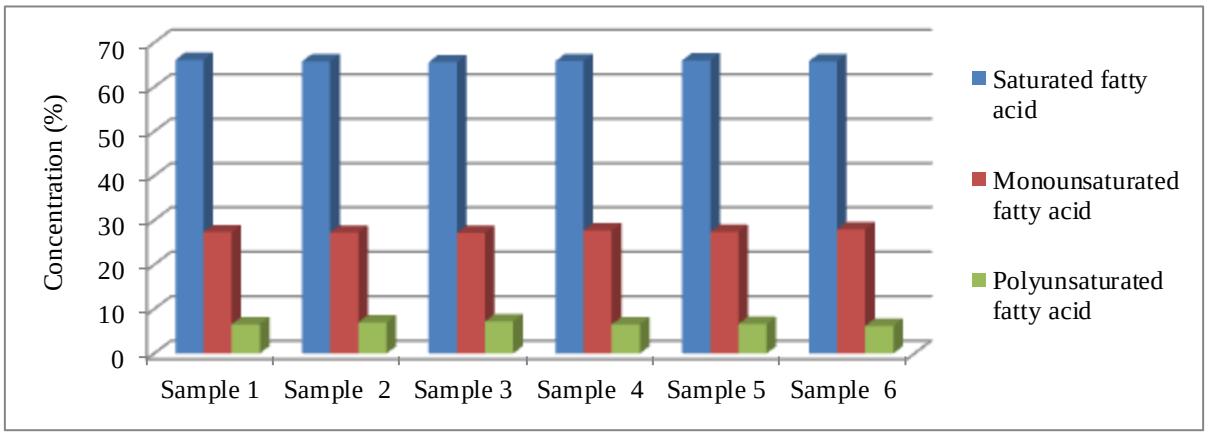


Figure 1. Fatty acids content in examined cheeses

Based on the data processing, it can be noticed that, for the content of most of the identified fatty acids in the examined cheeses, there are no statistically significant differences ($p > 0.05$) due to the conditions of milk coagulation. Of the saturated fatty acids in the tested cheeses, palmitic (C16:0), stearic (C18:0) and myristic (C14:0) acids are the most abundant (Table 2). The total content of palmitic acid ranged from 30.61% (sample 3) to 31.00% (sample 5). The content of stearic acid ranged from 11.57% (sample 3) to 11.79% (sample 5), and the content of myristic acid ranged from 10.89% (sample 3) to 10.98% (samples 5 and 6). Other identified saturated fatty acids were found in much smaller amounts in the tested samples. Oleic acid with a content of 24.05% (sample 3) to 24.40% (sample 6) is the most abundant fatty acid in the group of monounsaturated fatty acids (Table 3), which coincides with the data from the literature (Kinik et al., 2005; Vilušić et al., 2008; Kralj et al., 2015). The content of fatty acids in milk depends on the production method and nutrition and keeping of cows. Polyunsaturated fatty acids are very important for assessing the quality of milk and dairy products. Of polyunsaturated fatty acids in the tested cheeses, the largest amount was eicosadienoic acid (C20:2) in the range from 2.76% (sample 6) to 3.83% (sample 3) and linoleic acid (C18:2) in the range from 2.09% (sample 2) to 2.13% (sample 4) (Table 4).

The lack of fatty acids in food, such as linoleic acid, linolenic acid and other acids, can lead to various human diseases, which is why they belong to the group of essential fatty acids. Linoleic acid and linolenic acid cannot be synthesized by mammals and they must be taken into the organism with food. Linoleic acid belongs to the group of omega-6 acids and linolenic acid to the group of omega-3 fatty acids. Omega-6 and omega-3 fatty acids today are gaining more and more importance in human nutrition and are the subject of an increasing number of studies (Walstra et al., 2006; Kralj et al., 2015).

Differences in the composition of fatty acids in milk can also be the result of different breeds, seasons and the proportion of fat in milk (Popović-Vranješ et al., 2010). Kralj et al. (2015) state that oleic acid, which belongs to the group of omega-9 fatty acids, is the most abundant unsaturated fatty acid in milk from conventional production. From the group of omega-6 acids, significant are the contents of linoleic acid and gamma linolenic acid. The content of linolenic fatty acid, which belongs to the group of omega-3 fatty acids, is very important for the assessment of milk quality. Vilusic et al. (2008) found that palmitic acid (C16:0) was dominant in the fatty acid composition of fresh Quark cheese with 40.01%, which was 56.57% of the total saturated fatty acids of this sample. In the group of monounsaturated fatty acids, the most common was oleic acid, and the main polyunsaturated fatty acid in fresh cheese is linoleic acid (C18:2).

Table 2. Saturated fatty acids content in the examined cheese samples

Fatty acids	Quantity (%)					
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Butyric (C4:0)	1.87±0.14	1.74±0.47	1.91±0.02	1.88±0.14	1.55±0.31	1.50±0.26
Caproic (C6:0)	1.66±0.00	1.55±0.11	1.74±0.14	1.64±0.01	1.67±0.14	1.61±0.05
Caprylic (C8:0)	1.05±0.01	1.00±0.03	1.07±0.03	1.04±0.00	1.07±0.07	1.06±0.04
Capric (C10:0)	2.34±0.00	2.32±0.01	2.34±0.01	2.34±0.00	2.35±0.05	2.36±0.02
Undecanoic (C11:0)	0.03±0.00	0.03±0.00	0.03±0.00	0.09±0.00	0.03±0.00	0.03±0.00
Lauric (C12:0)	2.79±0.01	2.78±0.02	2.77±0.00	2.79±0.01	2.80±0.05	2.82±0.02
Tridecanoic (C13:0)	$0.11^{b.c}$±0.00	$0.10^{a.b.c}$±0.00	0.11^{c}±0.00	$0.10^{a.b}$±0.00	$0.10^{a.b.c}$±0.00	0.10^{a}±0.00
Myristic (C14:0)	10.96±0.02	10.94±0.07	10.89±0.03	10.91±0.05	10.98±0.15	10.98±0.04
Pentadecanoic (C15:0)	1.41±0.00	1.41±0.00	1.40±0.01	1.40±0.00	1.41±0.01	1.41±0.00
Palmitic (C16:0)	30.92±0.03	30.91±0.20	30.61±0.03	30.79±0.19	31.00±0.31	30.91±0.12
Heptadecanoic (C17:0)	0.92±0.00	0.93±0.00	0.94±0.01	0.91±0.01	0.93±0.01	0.91±0.01
Stearic (C18:0)	11.74±0.03	11.75±0.13	11.57±0.01	11.69±0.10	11.79±0.10	11.73±0.00
Arachidic (C20:0)	0.24±0.00	0.24±0.01	0.24±0.00	0.24±0.00	0.25±0.00	0.23±0.00
Ligonoceric (C24:0)	0.08±0.11	0.08±0.11	0.0000	0.0000	0.08±0.11	0.15±0.01

$^{a-c}$ mean values with different letters in the same column differ statically significantly with 95% probability ($p < 0.05$)

Table 3. Monounsaturated fatty acids content in the examined cheese samples

Fatty acids	Quantity (%)					
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Myristoleic (C14:1)	0.81±0.02	0.80±0.00	0.80±0.00	0.86±0.07	0.81±0.00	0.92±0.14
Cis-10-Pentadecenoic (C15:1)	0.06±0.01	0.06±0.002	0.06±0.01	0.25±0.28	0.05±0.01	0.25±0.28

Palmitoleic (C16:1)	1.71±0.03	1.70±0.00	1.69±0.01	1.70±0.00	1.70±0.02	1.71±0.00
Cis-10-Heptadecenoic (C17:1)	0.38±0.00	0.39±0.00	0.39±0.00	0.38±0.01	0.38±0.00	0.38±0.01
Oleic (C18:1 c+t)	24.12±0.03	24.09±0.37	24.05±0.14	24.22±0.20	24.21±0.23	24.40±0.01
Cis-11 Eicosenoic (C20:1)	0.23±0.00	0.19±0.09	0.16±0.00	0.17±0.10	0.20±0.08	0.20±0.04
Erucic (C22:1)	0.00	0.00	0.00	0.12±0.16	0.00	0.08±0.12

Table 4. Polyunsaturated fatty acids content in the examined cheese samples

Fatty acids	Quantity (%)					
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Linoleic (C18:2 c+t)	2.10±0.01	2.09±0.03	2.10±0.01	2.13±0.00	2.11±0.01	2.12±0.01
γ- Linolenic (C18:3 n6)	$0.16^{a.b}$±0.00	$0.16^{a.b}$±0.00	0.19^{b}±0.00	$0.17^{a.b}$±0.01	$0.16^{a.b}$±0.01	0.15^{a}±0.00
α- Linolenic (C18:3 n3)	0.58±0.00	0.58±0.00	0.58±0.00	0.59±0.01	0.59±0.00	0.59±0.00
Eicosadienoic (C20:2)	3.10±0.13	3.62±0.30	3.83±0.27	3.10±0.19	3.16±0.72	2.76±0.06
Eicosatrienoic (C20:3 n6)	0.17^{b}±0.03	$0.08^{a.b}$±0.07	$0.13^{a.b}$±0.02	$0.16^{a.b}$±0.02	0.03^{a}±0.01	$0.15^{a.b}$±0.01
Eicosatrienoic (C20:3 n3)	0.14±0.03	0.12±0.01	0.12±0.00	0.14±0.02	0.13±0.00	0.13±0.00
Eicosapentaenoic (C20:5)	0.18±0.12	0.17±0.12	0.18±0.13	0.17±0.13	0.26±0.04	0.24±0.02
Docosadienoic (C22:2)	0.15±0.03	0.17±0.14	0.08±0.10	0.07±0.10	0.20±0.20	0.08±0.11

[a-b] mean values with different letters in the same column differ statically significantly with 95% probability ($p < 0.05$)

Conclusions

Based on the obtained results, it can be concluded that coagulation conditions do not have a statistically significant effect ($p > 0.05$) on the content of majority of the identified fatty acids in acid-coagulated cheeses. A much higher content of saturated fatty acids than unsaturated fatty acids was found in the tested cheeses. The most common saturated fatty acids in the tested cheeses were palmitic (C16:0), stearic (C18:0) and myristic (C14:0) acid, and oleic acid (C18:1) was the most common unsaturated fatty acid. Of the total amount of identified fatty acids, the content of palmitic acid ranged from 30.61% (sample 3) to 31.00% (sample 5), the content of stearic acid from 11.57% (sample 3) to 11.79% , while the myristic acid content ranged from 10.89% (sample 3) to 10.98% (samples 5 and 6). Oleic acid was present in the tested cheeses in the amount of 24.05% (sample 3) to 24.40% (sample 6). Other identified fatty acids were present in smaller amounts.

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EFFECTS OF IBA CONCENTRATION ON PROPAGATION OF CHIMONANTHUS PRAECOX (L.) LINK BY SOFTWOOD CUTTINGS

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Abstract

This deciduous large shrub has very abundant yellow, strongly scented, flowers produced during winter. For that reason, it is valuable species for landscape use as an ornamental plant. Its vegetative propagation is usually done by spring cuttings or layering. The aim of this study was to determinate the optimal auxine concentration for rooting of softwood cuttings of selected elite genotype of *C. praecox* growing in the Belgrade area. Nodal softwood cuttings were used and treated with 25 ppm, 50 ppm or 100 ppm solution of IBA during 24 h, before sticking in sand. Six weeks after sticking, cutting were taken from substrate, examined and their state (callused, rooted or without change) was recorded. Rooting percentage was low, not exceeding 20%, but the number of callused cutting was higher than 50% in all treatments and control. Cuttings were returned to the rooting substrate for additional 6 weeks and after that the rooting percentage as well as number and length of primary and secondary roots were determined. Results obtained in a control treatment without IBA were poor (only 15.8% rooted cuttings), indicating that auxine treatment was necessary in a propagation of this species. Cuttings treated with 50 ppm IBA had the highest rooting percentage (34.7%), but the highest number of primary roots and the longest roots developed on cuttings treated with 100 ppm IBA. Taking into account that rooting of softwood cuttings can be strongly influenced by time of their collection, the obtained results could be improved by additional research.

Key words: *wintersweet, vegetative propagation, cuttings, greenwood, auxine.*

Introduction

Chimonanthus praecox (L) Link (Fam. Calycantaceae) is a 2 - 3 m tall deciduous shrub that flowers during winter from December to February, usually in January. The flowers are very abundant, yellow, strongly scented. Wintersweet is native in China, where is grown as a valuable ornamental and medicinal species, having importance as a cut flower and the source of a natural essential oil, which can be used in perfumery, cosmetics and aromatherapy (Liu *et al.,* 2014; Chen *et al.,* 2017). In Europe, wintersweet is grown mostly in parks and urban green areas (Vukićević, 1996; Kozomara *et al.,* 2008). Taking all these characteristics into account, *C. praecox* should be used more often in the green spaces and gardens in Serbia.

C. praecox can be propagated by seed but it takes 5-12 years from sowing to flowering (Macdonald, 1986; Lamb *et al.,* 1975). Its vegetative propagation is usually done by layering or green cuttings (Kozomara *et al.,* 2003; Grbić, 2004). There are also reports about micropropagation of *C. praecox* but this method is not widely used in mass production of this species (Wang and Shen, 1988; Kozomara *et al.,* 2003, 2008; Zhao *et al.,* 2012). Du *et al.* (2011) propagated wintersweet by grafting with a survival rate of 66.2%.

Although propagation by green cuttings has been recommended for *C. praecox*, there is no much reports about expected rooting rate. Kozomara *et al.* (2003) propagated *C. praecox* using green cuttings with 1-2 nodes, treated with 0.8 % IBA (indole-3-butyric acid) powder, and the highest rooting rate obtained in their research was 37.3%. Considering that cutting

size can affect rooting considerably (Marković *et al.,* 2017; Gerrakakis and Özkaya, 2005; Yang *et al.,* 2015), authors decided to research a possibility of using larger green cuttings with more than 2 nodes in propagation of this species. Also, different IBA application method was used, the cutting wer immersed in IBA solution instead of treatment with IBA powder preparation.

Material and Methods

Two shrubs of *C. praecox* growing in Arboretum of the Faculty of Forestry in Belgrade (Serbia) were selected as cuttings source. The green cuttings were taken on May, 24. The shoots were collected early in the morning, soft tips were removed, and cuttings with firm stems, with 3-4 internodes, were cut. Basal leaves were removed and the remaining leaves were cut in half to prevent cuttings from drying out. The cuttings were treated with 0.6% Captan® fungicide to prevent the development of pathogens. The base of cuttings was immersed in 20 ppm, 50 ppm or 100 ppm of IBA solution for 24 h at the temperature of 3-5°C, and at the same time, the cuttings in a control treatment were immersed in a distilled water. The cuttings were sticked in sand, in randomized block design, and rooting was performed under intermittent mist, in the greenhouse of the Faculty of Forestry, Belgrade. Three replications of 50 cuttings were used per treatment. Length of cuttings and number of internodes were recorded. The state of cutings and rooting percentage were determined 6 weeks after sticking. The cuttings (except necrotic ones) were put back in a rooting substrate for additional 6 weeks, and after that the rooting percentage, number and length of primary and secondary roots were measured.
The significance of differences between the means was calculated by the analysis of variance (ANOVA, $p < 0.05$) and the least significant difference (LSD) test.

Results and Discussion

The length of cuttings and number of internodes were recorded in order to avoid the influence of cuttings size on rooting. According to obtained data (Table 1), the average length of cuttings recorded in each treatment group ranged from 6.7 cm to 7.4 cm, while the average number of internodes ranged from 2.9 to 3.1. These differences were not statistically significant, so they did not influence the obtained results.

Table 1 The length of cuttings and number of internodes

Treatment	Cutting length (cm)	Number of internodes (%)
Control	6.8 a	2.9 a
20 ppm	6.7 a	3.1 a
50 ppm	7.0 a	2.9 a
100 ppm	7.4 a	3.1 a

Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

Six weeks after sticking, cutting were taken from substrate, examined and their state (callused, rooted, necrotic or without change) was recorded (Table 2). Rooting percentage was low, 5 - 20%, but the number of callused cutting was higher than 50% in all treatments and control. Almost a quarter of cuttings were necrotic (22.5-28.0%) and they were removed. After additional 6 weeks of rooting, the cuttings were measured again (Table 3) and small number of the new necrotic cuttings was recorded (6.7-16.7%).

Table 2 State of cuttings 6 weeks after sticking

Treatment	Callusing (%)	Unchanged (%)	Necrotic (%)	Rooted (%)
Control	64.2 a	8.3 b	22.5 a	5.0 c
20 ppm	59.3 a	2.7 ab	26.7 a	11.3 bc
50 ppm	51.3 a	1.3 a	27.3 a	20.0 a
100 ppm	55.3 a	0.7 a	28.0 a	16.0 ab

Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

Although the rooting percentage was still very low after 12 weeks, ranging from 15.8% to 34.7%, it increased considerably compared to the results recorded 6 weeks after sticking.

Table 3 State of cuttings 12 weeks after sticking

Treatment	Rooted (%)	Necrotic (%)	No of primary roots	Length of primary roots (cm)	No of secondary roots	Length of secondary roots (cm)
Control	15.8 b	6.7 b	4.3 b	12.8 b	10.0 a	1.0 a
20 ppm	28.0 a	8.7 b	3.7 b	11.2 b	13.3 a	1.7 a
50 ppm	34.7 a	10.0 ab	4.5 b	13.4 b	12.5 a	1.6 a
100 ppm	28.0 a	16.7 a	7.8 a	24.9 a	17.3 a	1.2 a

Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

IBA concentration influenced the number and length of primary roots, but statistically significant difference was recorded only when 100 ppm IBA treatment was applied. The number and length of secondary roots did not differ significantly from a control (Table 3).
The results obtained in this research were not satisfactory. However, they provide some additional data useful for a further research. Rooting percentage of 34.7% was recorded after 12 weeks, using cuttings with 3-4 nodes which were taken at the end of May. Kozomara *et al.* (2003) took cuttings in 4 different times (June 21, July 8, July 24 and August 8) and they recorded the highest rooting percentage of 37.3% after 6 weeks for cuttings taken on July 24th. However, in their research, the treatment with 24 h of cuttings immersion in 50 mg/L IBA solution resulted with only 23.9% rooted cuttings. Taking into account that time of taking cuttings can influence rooting rate (Marković *et al.*, 2014; Marković and Popović, 2012; Kosina and Baudyšová, 2011; Ayan *et al.*, 2006), there is a possibility that results obtained in our research could be better if the cuttings were taken lately in June or July. After 6 weeks the rooting percentage was only 20% which is lower than Kozomara *et al.* (2003) recorded also after 6 weeks. Positive effect of longer time period for rooting of cuttings has already been reported for some species (Haynes *et al.*, 2003) as well as the effect of cutting size on rooting (Gopale and Zunjarrao, 2011; Vigl and Rewald, 2014; Yang et al., 2015). However, in some cases cutting size did not affected rooting (Bona *et al.*, 2012; Owuor *et al.*, 2009) or the influence of cutting size on rooting rate depended on type of cuttings and auxine concentration (Marković *et al.*, 2017). IBA concentrations used in our research were relatively low compared to concentrations used by Kozomara *et al.* (2003) in a propagation of *C. praecox* and concentrations generally recommended for propagation of woody species using immersion of green cuttings for 24h in auxine solution (Hartmann *et al.*, 2014; Marković *et al.*, 2017). However, in some cases low concentrations of 10 ppm to 100 ppm of IBA or NAA can be effective in propagation of woody species, but auxine type influenced rooting

considerably (Topacoglu *et al.*, 2016). In our research the concentration of 50 ppm of IBA also resulted in a higher rooting rate than 100 ppm IBA, but primary roots were shorter. Besides, the very large number of callused cuttings can also be a reason for low rooting rate because sometimes excess callusing may obstruct rooting (Hartmann *et al.*, 2014).

Conclusions

Obtained results were not satisfactory, the best result was achieved with cuttings treated with 50 ppm IBA, where rooting was 34.7%. However, according to obtained results, and results published by other researchers, it can be concluded that our results can be significantly improved by investigating the influence of time of cuttings collection as well as the effect of other auxines on rooting of *C. praecox* cuttings. During these additional researches, the larger cuttings, containing 3-4 nodes, should be used.

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MORPHOLOGICAL CHARACTERISTICS OF AUTOCHTHONOUS GENOTYPES OF SWEET CHERRY (*PRUNUS AVIUM* L.) CV. 'ALICA' AND 'HRUST' IN AREA OF HERZEGOVINA

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Abstract

The paper presents the results of a two-year study of morphological characteristics of autochthonous sweet cherry (*Prunus avium* L.) varieties 'Alica' and 'Hrust' within the period 2018-2019 in Herzegovina region in Bosnia and Herzegovina.This study included monitoring of quantitative pomological characteristics such as fruit weight, width, length and thickness, stone weight fruit skin weight, fruit stem length leaf length and width, leaf stalk length, fruit shape index,as well as flesh percentage. On the basis of evaluated data, the best fruit performance was registered at some varieties of sweet cherry grown in a condition of this part of Herzegovina. The highest average of fruit weight (8.72 g), fruit width (25.18 mm) and fruit length (22.36 mm) was found in a variety of 'Hrust DH1'. As for fruit thickness, variety 'Hrust GH4' had the highest value (21.35 mm). The 'Alica' species showed lower values of the analyzed parameters, which could be related to the varietal characteristic because it belonged to the varieties with smaller fruit. The highest fruit weight, length, width, and t thickness was shown by 'Alica JA2' (6.32 g, 21.05 mm, 22.94 mm and 19.45 mm, respectively). Principal Component Analysis (PCA) shows reliability for separation of all analyzed autochthonous 'Alica' and 'Hrust' cherry genotypes based on morphological characteristics. The analysis of the obtained results shows that the total variance of the research is within the dominant eigenvectors in the first main component (PCA), which accounted for 40.795% of the research. The most significant properties of the first component relate to the morphometric properties of the cherry fruit. Within the second main component, which accounts for 56.968% of the total variability of the experiment, most of the properties with a high value for the eigenvector are related to the morphological characteristics of the leaves. Based on the research results, it may be concluded that this, once very respectable variety, over the years has acquired adaptability to climate conditions and that it should be further investigated as a valuable genetic potential.

Keywords: *autochthonous, genotypes, Alica, Hrust, sweet cherry.*

Introduction

The Balkan Peninsula is one of the most abundant centers of fruit species' genetic diversity in Europe. The old indigenous varieties and the abundance of natural populations of fruit species on the Balkan Peninsula should be the subject of comprehensive research work about its collection and study. The particular significance of these genotypes is reflected in the fact that they possess important economic-biological and pomological characteristics, which make them a suitable starting material for plant breeding. The study of the genetic diversity of the stone-fruit species populations has indicated the presence of unique or extremely rare traits that can significantly improve the economically most important characteristics of varieties and

rootstocks (Keserović *et al.*, 2017). Jarebica and Kurtović (1997) state that the most valuable genotypes will find their place in breeding programs, aiming to preserve units that currently have no production value. For these reasons, the preservation of germplasm should provide an opportunity to create new genotypes (varieties and rootstocks of fruit trees), which will be able to fulfill the fruit needs of contemporary and future generations of people (Mišić, 1987). Lately the autochthonous fruit species and varieties in Bosnia and Herzegovina have been increasingly researched because it can be said with certainty that the entire Balkan Peninsula is very rich in plant genetic resources (Aliman *et al.*, 2013; Skender *et al.*, 2014). Next to being a significant source of genetic variability, the genetic fund of autochthonous cherry genotypes (*Prunus avium L.)* can also serve as a highly valued starting material that can be used in subsequent research through breeding programs in order to improve the agronomic characteristics of this specie. The aim of this paper is to identify those genotypes that will stand out by their morphological characteristics in the area of Herzegovina, by analyzing the autochthonous genotypes of the two most common genotypes, 'Alica' and 'Hrust', and thus represent the basis for further genetic research for sustainable conservation and utilization of this important plant genetic resource of Herzegovina region.

Material and Methods

The trees marked for research were found in the Herzegovina-Neretva Canton in the area of: Mostar (Vrapčići, Salakovac, Humi, Hodbina, Gnojnice), Čapljina (Domanovići, Opličići, Bivolje brdo) and Jablanica (Slatina). Most of the selected trees were in the age of 30 and over. The geographic coordinates were determined for selected cherry genotypes. The 'Alica' and 'Hrust' cherry genotypes from one area were classified into a group and the labeling consisted of the group-locality prefix and the number of the sampled variety. The sample presented 30 genotypes of 'Alica' and 'Hrust' cherries. For morphological characterization, 30 fruits and leaves were taken from each tree. The phenotypic monitoring was performed *in situ*, i.e. *on-farm* conditions through two trial years (2018 and 2019). The morphological characterization of cherries at the phenotypic level was performed according to the guidelines included in the IBPGRI Cherry descriptor, published by the International Board for Plant Genetic Resources (1985) and the UPOV descriptor (UPOV, 2006), to describe species of the *Prunus avium* genus. The analysis of the results of the morphological characteristics' evaluation of 60 cherry genotypes in the mentioned areas was performed by applying the analysis of principal components (PCA - Principal Component Analysis) (Hotelling, 1936) based on the correlation matrix in the computer program R c. 3.2.3 (R core team, 2016). The principal components analysis (PCA) was performed using the mean values of 12 quantitative characteristics. The morphological measurements included measurements of quantitative fruit characteristics such as: fruit lenght (VP), width (SP) and fruit thickness (DP), fruit stem length (DPP), fruit weight (MP), stone weight (MK), weight of fruit skin (MPP), flesh percentage (RM) and fruit shape index (IOP). The analyzed leaf characteristics are: leaf length (DL), leaf width (SL) and leaf stalk length (DPL). The combinations of modalities of experimental factors based on the first two components are presented graphically in the form of a spatial distribution of analyzed cherry genotypes. The main components explain the variability of the data in a concise way as well as the interrelationships of the variables.

Results and Discussion

After conducting the experimental work on two autochthonous cherry varieties 'Alica' and 'Hrust' in the area of Herzegovina through two trial years (2018-2019), the following results are presented

Table 1. The average values with standard deviation of 12 morphological parameters of identified perspective 'Alica' cherry genotypes for two experimental years

GENOTYPE	FRUIT LENGTH	FRUIT WIDTH	FRUIT THICKNESS	STONE WEIGHT	WEIGHT OF FRUIT SKIN	FRUIT STEM LENGTH	FRUIT WEIGHT	LEAF LENGTH	LEAF WIDTH	LEAF STALK LENGTH	FRUIT SHAPE INDEX	RM	LATITUDE LONGITUDE	LATITUDE LONGITUDE
HOA1	17.83±2.58	16.39±2.59	14.77±2.08	**0.29±0.05**	0.35±0.07	3.88±0.49	3.28±0.52	12.16±1.30	6.48±0.57	4.23±0.57	**1.31**	89.25	4314096	1751327
HOA2	18.65±2.99	18.58±2.87	15.48±2.89	0.42±0.04	0.36±0.03	3.95±0.49	4.40±0.95	11.13±1.80	6.61±1.19	**3.26±1.19**	1.21	91.72	4313281	1751415
HOA3	17.52±1.36	18.05±1.00	16.02±1.09	0.32±0.04	0.43±0.03	3.30±0.45	**3.20±0.44**	12.55±1.51	5.74±0.68	3.75±0.68	1.06	86.56	4313298	1751513
HOA4	18.23±2.80	18.25±2.95	15.62±2.41	0.36±0.06	0.31±0.05	3.54±0.44	4.19±0.85	11.62±1.03	5.68±0.62	3.57±0.62	1.17	92.55	4313428	1751598
VA1	19.45±2.50	19.49±2.52	17.43±2.80	0.38±0.06	0.50±0.04	4.02±0.30	5.75±0.91	10.76±0.93	5.76±0.45	3.72±0.45	1.11	91.24	4337615	1786335
VA2	17.62±1.66	17.22±2.42	14.61±2.35	0.37±0.06	0.49±0.03	3.63±0.24	3.51±0.60	11.99±1.28	6.39±0.56	4.17±0.56	1.23	**85.95**	4337605	1786338
VA3	18.75±0.77	18.77±0.95	16.46±0.73	0.31±0.04	0.33±0.03	3.98±0.33	3.55±0.42	10.81±1.19	5.52±0.69	3.62±0.69	1.14	90.54	4337679	1785979
VA4	18.32±1.40	18.28±1.13	16.75±1.22	0.38±0.03	0.49±0.04	3.82±0.32	4.62±0.52	11.30±0.82	6.06±0.47	3.78±0.47	1.10	89.40	4337633	1785989
VA5	17.46±1.72	17.47±1.74	15.32±2.49	0.36±0.05	0.32±0.06	3.79±0.28	4.18±0.58	10.08±0.95	4.97±0.65	3.82±0.65	1.14	92.20	4337614	1786313
HA1	18.45±1.55	19.17±2.17	16.65±1.52	0.31±0.04	0.44±0.05	**3.29±0.37**	4.30±0.60	11.56±1.40	5.33±0.68	3.82±0.68	1.07	89.70	4344385	1790004
HA2	19.31±2.87	18.78±2.51	16.38±2.26	0.30±0.04	**0.24±0.05**	3.54±0.35	4.03±1.05	10.31±0.46	4.82±0.55	3.83±0.55	1.21	**93.88**	4344384	1789993
HA3	17.03±1.59	19.99±2.22	17.57±1.91	0.34±0.05	0.33±0.06	3.36±0.40	4.82±0.91	10.20±0.47	4.77±0.55	4.18±0.55	0.83	93.02	4344364	1789997
SA1	20.95±1.50	21.77±1.88	18.67±1.36	0.43±0.09	0.48±0.06	3.86±0.63	5.46±1.01	11.15±1.08	6.19±0.70	3.38±0.70	1.08	91.18	4344351	1785646
SA2	16.48±1.23	**16.38±1.28**	**14.06±1.25**	0.39±0.09	0.36±0.02	3.30±0.29	3.73±0.44	**13.24±1.16**	6.58±1.00	4.18±1.00	1.18	90.14	4343949	1785644
SA3	18.64±1.48	18.79±1.63	16.53±2.85	0.35±0.04	**0.57±0.04**	3.96±0.41	4.98±0.71	10.09±1.20	4.95±0.89	3.76±0.89	1.12	88.58	4343943	1785593
SA4	16.99±1.54	16.95±1.86	15.18±2.51	0.35±0.05	0.40±0.05	3.56±0.30	3.73±0.59	11.58±1.63	6.18±0.76	**4.53±0.76**	1.12	89.31	4344285	1785732
SA5	17.35±1.19	17.24±1.30	14.79±1.71	0.36±0.04	0.46±0.05	3.86±0.25	4.38±1.35	10.40±0.65	4.91±0.52	3.66±0.52	1.18	89.47	4344286	1785738
SA6	17.69±1.73	17.34±2.07	15.02±2.45	0.38±0.04	0.36±0.03	3.58±0.15	4.09±0.80	10.69±0.97	**4.53±0.60**	3.75±0.60	1.20	91.15	4344288	1785743
DA1	17.89±1.15	21.91±1.24	18.44±2.36	0.43±0.05	0.42±0.03	3.88±0.47	5.44±0.53	12.36±1.39	**6.84±1.20**	4.02±1.20	1.12	92.18	4308256	4347403
DA2	18.42±1.41	17.94±1.40	16.17±1.74	0.38±0.07	0.34±0.04	3.86±0.50	4.61±0.58	**9.74±0.77**	5.09±0.51	4.00±0.51	1.17	92.54	4308266	4347405
DA3	17.93±0.94	17.82±1.01	15.82±0.77	0.36±0.04	0.35±0.04	3.60±0.41	3.69±0.38	11.36±0.94	5.92±0.74	3.85±0.74	1.14	90.38	4307574	1747190
DA4	17.56±1.68	16.77±1.56	15.04±1.79	0.38±0.05	0.35±0.05	3.35±0.41	4.00±0.60	10.60±0.49	4.96±0.57	3.88±0.57	1.22	91.13	4308108	1754011
DA5	**16.46±1.18**	19.45±1.72	17.16±1.22	0.34±0.06	0.35±0.06	3.44±0.43	4.06±0.63	11.89±0.77	5.90±0.86	3.83±0.86	**0.81**	91.36	4307114	1748469
BBA1	19.26±1.04	19.54±1.57	17.33±0.88	0.38±0.04	0.36±0.04	**4.56±0.57**	4.29±0.52	10.15±0.91	5.28±0.71	3.28±0.71	1.10	91.53	4314659	1773306
BBA2	20.02±1.30	20.55±2.11	17.30±2.03	0.49±0.09	0.40±0.03	4.37±0.67	5.96±0.61	10.42±0.92	5.23±0.49	4.03±0.49	1.13	93.22	4314653	1773319
JA1	19.61±0.86	20.81±0.96	17.80±0.92	**0.50±0.05**	0.38±0.05	3.86±0.35	5.87±0.82	11.87±0.77	5.89±0.86	3.83±0.86	1.04	93.49	4370002	1772988
JA2	**21.05±1.26**	**22.94±1.37**	**19.45±1.05**	0.45±0.06	0.41±0.03	4.51±0.63	**6.32±1.01**	10.98±1.02	5.39±0.57	3.46±0.57	0.99	93.49	4369999	1772989
JA3	19.29±0.79	18.45±2.49	16.97±0.81	0.32±0.02	0.37±0.05	3.50±0.23	4.47±0.50	9.90±0.45	4.63±0.53	3.68±0.53	1.19	91.67	4369993	1773000
JA4	19.93±1.04	20.41±1.00	18.59±1.03	0.38±0.06	0.44±0.03	3.92±0.23	5.77±0.57	11.74±1.04	5.75±0.63	3.61±0.63	1.05	92.31	4369983	1772974
JA5	20.39±1.14	20.94±1.16	18.71±1.11	0.41±0.05	0.38±0.04	4.34±0.47	5.92±0.81	12.76±1.56	6.23±0.88	4.16±0.88	1.06	93.56	4369971	1772977

Table 2. The average values with standard deviation of 12 morphological parameters of identified perspective cherry genotypes of 'Hrust' for two experimental years

GENOTYPE	FRUIT LENGTH	FRUIT WIDTH	FRUIT THICKNESS	STONE WEIGHT	WEIGHT FRUIT SKIN	FRUIT STEM LENGTH	FRUIT WEIGHT	LEAF LENGTH	LEAF WIDTH	LEAF STALK LENGTH	FRUIT SHAPE INDEX	RM	LATITUDE LONGITUDE	LATITUDE LONGITUDE
HOH1	18.81±2.28	19.95±3.14	16.24±2.86	0.45±0.06	0.50±0.04	3.87±0.39	4.74±0.87	11.82±1.89	6.76±0.58	3.93±1.04	**1.09**	89.42	4313281	1751415
HOH2	20.06±0.88	21.50±1.28	19.24±0.65	0.43±0.05	0.50±0.05	**4.98±5.10**	4.97±0.62	10.86±1.35	5.79±0.64	3.67±0.73	0.97	89.76	4314095	1751327
HOH3	18.80±2.56	20.18±3.20	17.25±2.92	0.51±0.04	0.65±0.03	3.76±0.64	4.94±0.89	12.10±0.99	6.65±0.54	3.56±0.69	1.02	**86.69**	4313281	1751415
HOH4	**17.48±1.86**	**18.50±2.21**	15.97±2.21	0.47±0.06	0.46±0.03	4.04±0.48	4.67±1.00	11.15±0.81	6.05±0.52	3.06±0.54	1.03	90.13	4313295	1751425
VH1	21.68±1.01	24.21±1.42	20.10±1.14	0.44±0.07	0.57±0.04	4.48±1.40	6.26±0.84	11.37±0.99	6.17±0.55	4.04±0.64	0.97	90.84	4337609	1786361
VH2	21.32±1.34	22.85±1.21	20.00±0.97	0.40±0.04	0.65±0.05	4.65±0.50	7.09±0.73	**12.72±1.05**	5.90±0.60	3.78±0.59	0.99	90.71	4337621	1786347
VH3	20.47±0.99	21.14±0.97	18.99±0.90	**0.58±0.04**	0.50±0.01	4.42±0.48	5.96±0.71	11.60±1.63	5.91±1.14	3.54±0.92	1.04	91.41	4337591	1786206
VH4	21.82±2.13	23.27±2.38	20.16±1.79	0.41±0.04	0.58±0.04	4.05±0.47	6.91±1.22	10.80±1.12	5.39±0.77	3.61±0.60	1.01	91.58	4337573	1786358
VH5	21.07±0.70	22.88±1.09	20.19±1.29	0.38±0.04	0.64±0.03	4.62±0.52	7.21±0.66	11.41±1.19	5.06±0.92	3.47±0.77	0.96	91.00	4337579	1786344
VH6	20.69±3.25	23.14±2.61	19.39±2.54	0.48±0.09	0.49±0.05	4.43±0.50	7.09±1.07	10.38±1.94	6.76±0.80	**4.27±1.14**	0.95	93.03	4337601	1786139
HH1	17.94±0.84	18.93±1.06	**16.31±0.88**	0.39±0.06	0.41±0.09	3.97±0.61	4.23±0.40	11.54±1.32	5.90±0.54	4.16±0.80	1.04	90.21	4344391	1790021
HH2	18.54±0.84	19.06±2.54	16.64±2.26	0.36±0.04	0.38±0.03	3.80±0.76	**3.70±0.40**	12.02±0.63	5.87±0.74	3.50±0.54	1.08	89.66	4344395	1790045
HH3	18.75±0.95	19.78±1.20	17.06±0.78	0.40±0.06	**0.36±0.05**	4.12±0.53	5.01±0.57	12.04±1.06	6.99±0.61	3.75±0.74	1.04	92.79	4344385	1789970
HH4	18.84±1.14	19.81±1.28	16.85±0.90	0.35±0.05	0.37±0.05	3.72±0.45	4.47±0.60	10.07±1.10	5.88±0.59	3.11±0.61	1.06	91.56	4344378	1789959
HH5	19.58±1.43	21.16±2.24	18.59±0.90	0.38±0.03	0.61±0.04	3.95±0.37	5.77±0.78	**9.77±0.60**	5.69±0.58	3.39±0.52	0.97	89.34	4344378	1789959
SH1	21.64±1.28	22.27±1.58	19.07±1.16	0.44±0.06	0.53±0.04	4.01±0.65	5.59±0.71	10.17±1.07	5.98±0.73	3.26±0.66	1.10	90.49	4343960	1785610
SH2	20.33±2.70	21.96±1.45	19.36±1.11	0.43±0.36	0.44±0.06	4.67±0.52	7.19±6.36	10.06±1.39	5.08±0.67	2.99±0.92	0.97	**93.87**	4343975	1785619
SH3	20.31±0.94	21.65±1.49	18.59±1.24	0.36±0.06	0.49±0.03	4.13±0.56	5.05±0.77	9.79±1.10	5.25±0.83	3.52±0.58	1.03	90.31	4343973	1785627
GH1	19.24±2.17	20.32±2.24	17.19±2.14	0.44±0.05	0.43±0.06	3.87±0.49	5.44±0.79	10.77±0.98	5.57±1.04	3.57±0.55	1.06	92.05	4317050	1751255
GH2	19.30±1.97	21.41±1.82	17.80±1.47	0.45±0.06	0.55±0.05	3.88±0.43	5.82±0.66	10.29±1.04	5.61±0.69	**2.96±0.58**	0.98	90.53	4317050	1751255
GH3	20.70±1.35	22.43±1.68	21.08±1.23	0.42±0.07	0.430.05	3.92±0.58	5.85±0.56	12.01±2.46	**7.12±0.82**	3.52±0.84	**0.91**	92.62	4317059	1752844
GH4	20.43±1.44	22.89±1.62	**21.35±0.76**	**0.33±0.03**	0.53±0.05	4.39±0.80	5.91±0.61	10.70±1.09	4.89±0.80	4.19±0.67	0.96	91.02	4318059	1775868
DH1	**22.36±1.68**	**25.18±1.30**	21.04±3.66	0.48±0.06	0.64±0.02	4.71±0.57	**8.72±1.06**	12.00±2.37	5.90±0.84	3.85±1.28	0.94	92.64	4307114	1748469
DH2	21.31±0.99	23.65±1.67	20.67±1.35	0.44±0.06	**0.67±0.01**	4.85±0.34	7.75±2.22	10.38±1.41	5.80±1.28	2.98±0.70	0.93	91.28	4307292	1751341
DH3	21.01±1.23	22.06±1.28	18.94±0.89	0.46±0.07	0.47±0.05	4.51±0.63	5.59±0.78	10.69±1.24	5.79±0.58	3.34±0.53	1.06	91.49	4308107	1751437
DH4	21.62±1.15	24.82±1.63	20.41±1.09	0.47±0.13	0.55±0.02	4.07±0.50	7.67±0.79	9.97±0.96	**4.89±0.66**	3.55±0.58	0.92	92.78	4306024	1753391
DH5	20.99±1.07	23.64±1.97	20.49±1.77	0.40±0.10	0.57±0.03	4.38±0.73	7.36±1.30	11.60±1.27	5.49±0.70	3.76±0.83	**0.91**	92.19	4307114	1748469
BBH1	19.44±1.89	20.15±1.78	17.14±1.54	0.42±0.10	0.45±0.04	**3.55±2.40**	5.30±0.91	12.26±1.34	6.01±0.62	4.26±0.82	1.09	91.35	4314611	1773367
BBH2	17.57±1.65	19.58±1.89	16.42±1.90	0.38±0.12	0.59±0.02	3.61±0.46	5.45±0.67	10.25±1.24	5.66±0.61	3.06±0.62	0.96	89.06	4314613	1773373
JH1	18.68±1.22	19.95±1.38	17.48±0.91	0.34±0.03	0.52±0.03	4.55±0.59	4.92±0.48	10.26±0.95	5.59±0.92	3.68±0.61	1.00	89.47	4370028	1772863

Analyzing the values of morphological parameters (Tables 1 and 2), the sample DH1 has been characterized by the highest average value of the fruit lenght (22.36 mm) and width (25.18 mm), while the lowest values of these parameters were typical for the DA5 (fruit lenght 16.46 mm) and SA2 (fruit width 16.38 mm). The average fruit thickness ranged from 21.35 mm (GH4 sample) to (14.06 mm) SA2 sample. The highest average fruit weight was recorded in the DH1 sample (8.72 g), while the lowest average fruit weight was in the HOA3 sample (3.20 g). The average stone mass ranged from (0.29 g) HOA1 sample to (0.58 g) VH3 sample. The lowest average value of the skin mass parameter was in the HA2 sample (0.24 g), while the highest average skin mass was measured in the DH2 sample (0.67 g). The identified values of the fruit shape index ranged from (0.81) DA4 sample to (1.31) HOA1 sample. The highest average value of fruit stem length was recorded in the HOH2 sample (4.98 cm), while the lowest average value was recorded in the HA1 sample (3.29 cm).

Analyzing the morphological characteristics of the leaves, it can be concluded that the highest average leaf length was recorded in the SA2 sample (13.24 cm), while the lowest average value of this parameter was recorded in the DA2 sample (9.74 cm). The average leaf width ranged from (4.53 cm) in the SA6 sample to (7.12 cm) in the GH3 sample. The identified value of the leaf stalk length ranged from (2.96 cm) GH2 sample to (4.53 cm) SA4 sample.

Hadžiabulić *et al.* (2017) state in the study conducted in 2013 and 2014 that the average fruit weight of 'Alica' ranged from (3.86 g) to (4.36 g), the stone weight ranged from (0.32 g) to (0.38 g), and the average fruit skin weight went from (0.28 g) up to (0.26 g). The flesh percentage ranged from 83% to 85%. On the basis of this research, it can be concluded that these are genotypes of 'Alica' cherry from the area in Herzegovina, which confirms the relevance of this research. In another study by Aliman *et al.* (2010) there were presented the morphological characteristics of the indigenous genotypes of the 'Alica' and 'Hrust' cherries in the period 2006-2007 in the adjaceny of Mostar. The results show that the autochthonous 'Hrus't cherry genotypes showed higher values of all morphological parameters compared to the 'Alica' genotypes, which is evident and confirmed in studies by other authors. The average fruit weight of the indigenous 'Hrust' cherry genotype was (6.08 g) and in the 'Alica' genotypes (5.19 g). The average stone mass varied from (0.41 g) in 'Hrust' to (0.36 g) in 'Alica'. The flesh percentage ranged from 92.90% for 'Hrust' to 92.68% for 'Alica'. The length of the fruit's stalk in the mentioned research was between (4.92 cm) ('Hrust') and (3.61 cm) 'Alica'. The study by Maglakelidze *et al.* (2015) presents the phenological and pomological characteristics of nine introduced cherry cultivars in Georgia through the period from 2014-2016. The study included cultivars 'Early Lory', 'Burlat', 'Moro', 'Lory Strong', 'Samba', 'Giorgia', 'Celeste', 'Krupnoplodnaja', and 'Van'. The fruit weight of the analyzed cultivars ranged from 6.9 g ('Moro') to 10.2 g ('Celeste'). The longest stalk was in the 'Lory Strong' (4.8 cm) and in the 'Giorgia' (4.6 cm) cultivars. The average stone weight was 0.43 g. Mratinić *et al.* (2012) in the study of genetic and morphological characteristics of 10 wild cherry genotypes have reached the result that the fruit weight varied from (0.78 g) in genotype 10, to (1.39 g) in genotype 5. The average fruit lenght varied from (0.95 cm) in genotype 10 to (1.18 cm) in genotype 1, and the average fruit width was between (0.89 cm) in genotype 8 and (1.13 cm) in genotype 1. Similar results were obtained by Karlidag *et al.*, (2009), for some wild sweet and sour cherry genotypes where the fruit mass was ranged from (0.76 g) to (2.11 g).

Table 3. Eigenvalues, proportion of variance and cumulative variance associated with the initial five principal components (PCA), estimated from a 12-variable correlation matrix in 60 autochthonus cherry genotypes

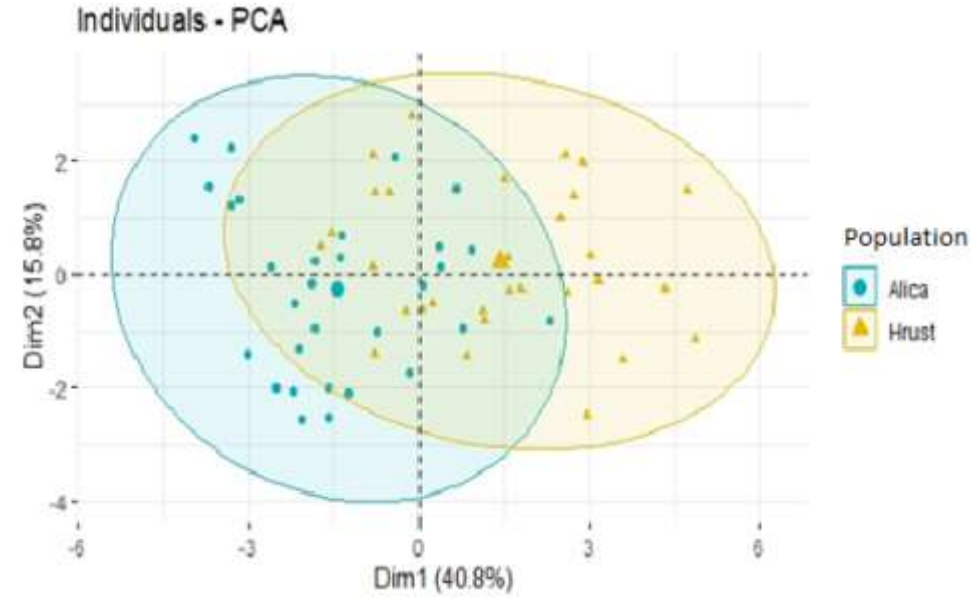
VARIBLES	PC1	PC2	PC3	PC4	PC5
EIGENVALUE	4.895	1.896	1.448	1.291	0.683
PROPORTION OF VARIANCE (%)	40.795	15.802	12.071	10.761	5.688
CUMULATIVE VARIANCE (%)	40.795	56.598	68.668	79.429	85.117

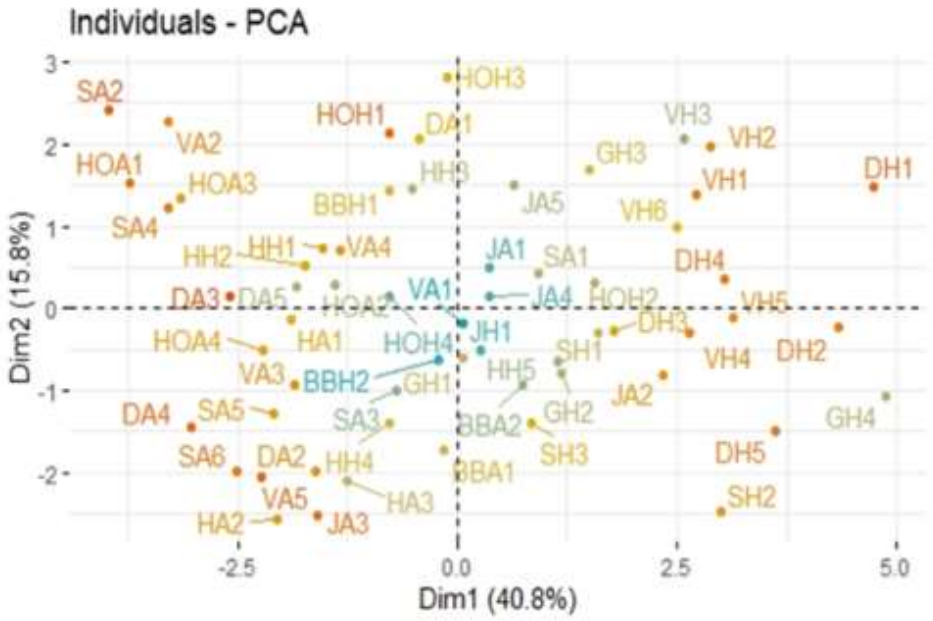
Table 4. Analysis of 12 quantitative characteristics of autochthonus cherry genotypes in total experimental variability (significant sources of variability are in bold)

	PCA1	PCA2	PCA3	PCA4	PCA5
FRUIT LENGTH – VP	**0.400**	0.017	-0.143	0.082	-0.272
FRUIT WIDTH – SP	**0.428**	0.062	-0.005	0.064	-0.156
FRUIT THICKNESS – DP	0.277	-0.049	**0.569**	0.052	0.262
STONE WEIGHT – MK	0.183	0.239	-0.378	0.110	**0.781**
SKIN MASS – MPP	0.309	0.207	0.017	**-0.528**	-0.146
FRUIT STEM LENGTH – DPP	**0.359**	0.033	-0.145	-0.004	-0.124
FRUIT MASS – MP	**0.418**	0.027	-0.107	0.074	-0.185
LEAF LENGTH – DL	-0.081	**0.615**	0.104	0.203	-0.082
LEAF WIDTH – SL	-0.021	**0.616**	-0.117	0.070	-0.027
LEAF STALK LENGTH – DPL	-0.148	0.257	**0.410**	0.367	-0.216
FRUIT SHAPE INDEX – IOP	-0.301	0.023	**-0.514**	0.026	-0.311
FLESH PERCENTAGE- RM	0.167	-0.259	-0.154	**0.713**	-0.035

Analyzing the results of the five principal components of the PCA analysis shown in Table 3, one can see the contribution of each of the 12 analyzed characteristic in the total variability present in the analyzed set of genotypes. Each of the 12 observed characteristics is found with a high value of eigenvectors in one of the first five principal components. The variables with the highest values of eigenvectors in the first five main components are presented: PC1 - fruit lenght, fruit width, fruit stem length, fruit weight; PC2 - leaf length and leaf width; PC3 - leaf stalk length and fruit shape index; PC4 - skin mass and flesh percentage and PC5 - stone weight. The analysis of the obtained results shows that the total variance of the research is within the dominant eigenvectors in the first main component (PCA), which accounted for 40.795% of the research. The most significant properties of the first component relate to the morphometric properties of the cherry fruit. The highest values of eigenvectors were in fruit weight and fruit width (0.418 and 0.428). The lower values of eigenvectors had the property of fruit lenght (0.400), while the lowest eigenvalue of vectors had the length of fruit stalk (0.359). Within the second main component, which accounts for 56.968% of the total variability of the experiment, most of the properties with a high value for the eigenvector are related to the morphological characteristics of the leaves. Of these, the properties with the highest value of eigenvectors are leaf width and length (0.616 and 0.615). Due to the visualization of the level of statistical significance of individual genotypes' separation and groups of autochthonus cherry genotypes in the first three graphs, the ellipsoids were placed

where the analyzed autochthonus cherry genotypes were classified into appropriate populations according to varietal affiliation with 95% reliability. The result of the association of the stated autochthonus cherry genotypes within individual populations is the presentation of the dispersion of genotypes in the mentioned populations based on the values of the afore-mentioned original variables.





Figures 1 and 2. Grouping of 60 autochthonus cherry genotypes in relation to the first two principal components (PC1 and PC2), calculated via a correlation matrix for 12 morphological traits

Figures 1 and 2 show the distribution of 60 autochthonus cherry genotypes obtained using the first two principal components calculated via a correlation matrix for 12 morphological traits, and it can be concluded that no separation of groups into separate groups was observed.
Analyzing the first two components, which contain 56.598% of the total variability of the experiment, it is evident that all groups of autochthonus cherry genotypes were grouped

mostly around the center of the coordinate system. The graph shows that there was a partial extent of overlap between the analyzed autochthonus cherry genotypes, which may indicate the fact that over time there was an exchange of genetic material, i.e. confirms the anthropogenic impact in terms of retention and spread of autochthonus cherry genotypes in Herzegovina.

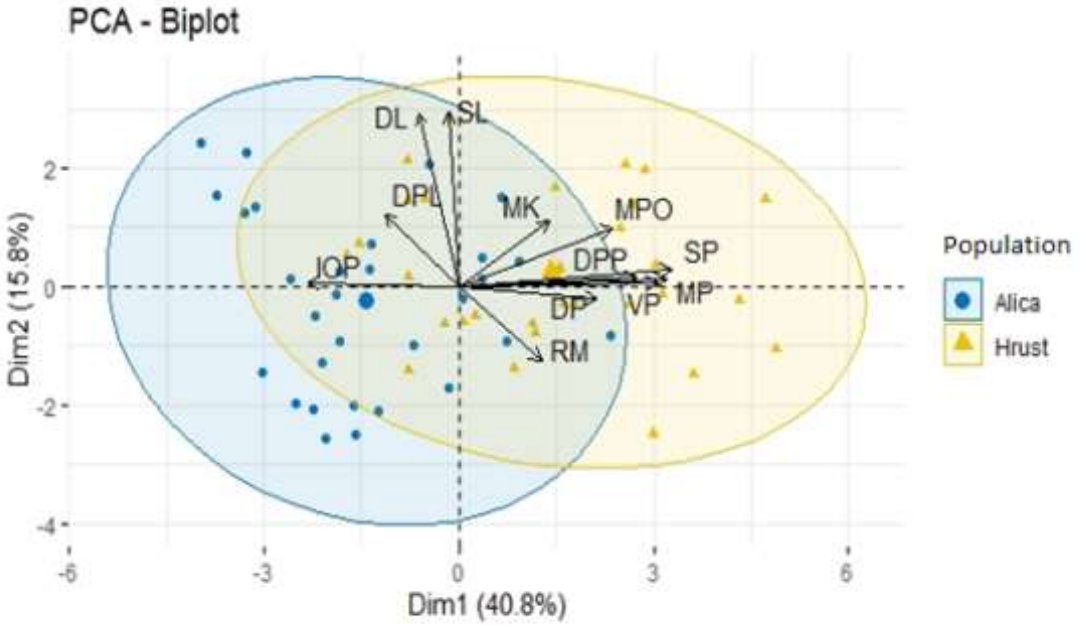


Figure 3. A biplot of 60 autochthonus cherry genotypes separated on the basis of morphological traits by the principal components analysis. The original properties are shown as vectors where the direction indicates the value for each property and the length to the level of variability

In addition to showing the separation of the analyzed cherry genotypes, Figure 3 of the biplot also includes an analytical presentation of the original analyzed characteristicss. From Figure 3, it can be seen that there is a strong positive correlation between fruit lenght, fruit width, fruit thickness, fruit stem length, fruit weight, stone weight, skin weight and flesh yield. The obtained result has a completely logical explanation in that the fruit mass and the core mass, as well as all other mentioned morphological characteristics, increase proportionally with the development of the fruit. A strong positive correlation was observed in the monitored morphological characteristics of leaf width, leaf length and leaf stalk length, and in this case the result is quite logical, since increasing leaf width and length implies increasing leaf area. The morphological feature of the fruit shape index showed a correlation with all morphological characteristics of the fruit, except with the properties of the leaves. The presence of a negative correlation was observed between pomological traits (flesh yield and fruit thickness).

Conclusions

Based on the research of morphological characteristics of autochthonous genotypes of 'Alica' and 'Hrust' cherries in the wider area of Herzegovina, from Jablanica to Stolac and Čapljina, in the period from 2018 to 2019, the following conclusions can be reported:

By analyzing the average value of morphological characteristics of fruit weight and stone weight, it can be concluded that the autochthonous cherry genotype 'Hrust' showed

statistically significant differences compared to the genotype 'Alica', which confirms the fact that 'Hrust' is a genotype of a larger fruit. As a conclusion of this research, it can be stated that certain genotypes of autochthonous cherry genotypes are characterized by one or more positive traits, and it is recommended to work on their more detailed evaluation and genetic characterization in order to preserve autochthonous materials to ensure the breeding of these species.

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VARIETY AND QUANTITY OF SEEDS AS FACTORS AFFECTING YIELD AND YIELD COMPONENTS OF WINTER TRITICALE

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Abstract

This paper analyzes the influence of different quantities of seeds (300 and 360 kg ha^{-1}) on the yield and yield components in 5 varieties of winter tricale (Odissey, Kg-20, Triumph, Rtanj and Tango) grown in the northern part of Montenegro during two growing seasons (2010/11 and 2011/12) on river alluvium soil type. During the trial, in both years of testing, all varieties were fertilized with the equal amount of NPK fertilizer in a ratio of 90:80:80. It was found that all of the three individual factors: variety, quantity of seeds and climatic conditions in the years of testing had a significant influence on the yield and other observed parameters. The test results showed that, on average, for all cultivars and both applied quantities of seeds, the highest yield was obtained with the Tango variety, while the lowest with the Kg-20 variety. It was also observed that the hectolitre weight in the tested triticale varieties were higher when applying a higher quantity of seeds. The application of a smaller quantity of seeds led to a significant increase in the 1000 grain mass in all cultivated varieties compared to the application of a higher quantity of seeds and it was also higher in the second study year.

Keywords: *Triticale, variety, quantity of seeds, fertility*

Introduction

Among other small grain cereals and forage crops, triticale takes a very important place because triticale has a great potential use for different purposes. Thanks to a high and stable yields, early maturity, good grain quality, resistance to lodging, good resistance to diseases, pests, cold and drought, triticale is suitable for cultivation at high altitudes, in soil with poor physical and chemical properties, such as acidic and saline soils (Villegas et al., 2010). Also, triticale is suitable for cultivation in mountain areas, where even the application of lower growing technology achieves satisfactory yields which means that triticale has cultivation opportunities even in areas where wheat production is debatable. Since its nutritional value is greater than the nutritional value of corn, as well as containing more protein and essential amino acids lysine than wheat, triticale is recommended by experts for nutrition in all species of domestic animals (Đekić et al. 2012; Biberdžić et al.2012), while Marković et al. (2016) point to the possibility of a complete or partial replacement of wheat and maize with triticale in mixtures for the consumption of consumable fish (carp). Due to all the above, triticale has become popular in recent years for growing on less fertile, degraded soils and soils with low pH value, which according to Biberdžić et al. (2012) represents a serious problem for field production because they are characterized by poor water-air and physical-mechanical properties, which makes production on them unstable. In addition to the variety, the sowing norm has a significant impact on triticale yield. In winter varieties, freezing of young plants is common, as well as lower germination energy and germination compared to other cereals (Milovanović et al., 1995). The quantity of seeds directly affects the number of ears per m^2, and indirectly changes other components such as the number of grains per ear and the 1000

grains mass (Mladenov et al., 2008). To achieve high yields and quality of triticale grains on soils with limited production capacity, such as acidic, the choice of the appropriate variety as well as the appropriate agrotechnics is of special importance. The aim of the study was to analyze the yield and components of grain yield in 5 varieties of winter triticale in the north of Montenegro, using two different sowing norms.

Material and Methods

The experiment was conducted with five cultivars of winter triticale (Odysey, Kg-20, Triumph, Rtanj and Tango) during the two growing seasons (2010/11 and 2011/12) in the area of Bijelo Polje (north Montenegro). The trials were set on the alluvium soil type, in random complete block design with four repetitions and experimental plot size of 6 m^2. Each plot consisted 16 rows with 12.5 cm spacing. Common technology for the production of triticale was used in the experiment. Before the basic land cultivation, mineral fertilizers in combination NPK 90:80:80 per ha were distributed on the soil surface and then ploughed in. Basic land cultivation was carried out in a classic way, on 25 cm depth. Complex NPK fertilizer (15:15:15) was applied while Calcium-ammonium-nitrate (KAN – 27% N). was applied as other nitrogen fertilizer in early spring. Sowing was carried out manually during the last decade of October, using two quantities of seeds (300 and 360 kg ha^{-1} of seeds). Grain yield was determined after the harvest which was conducted manually too. Upon harvest, the grain yield of each plot was measured and calculated as t ha^{-1} and corrected for 14% moisture. In addition to the yield, absolute and hectoliter mass were monitored. The average results of the yield of dry grain, hectoliter weight and 1000 grain mass are presented in this paper, for the analyzed period of two growing seasons. The obtained results were processed by the variance analysis method using the WASP 2.0 statistical package.

Soil and climatic conditions

Before setting up the experiments, soil samples from particular plots were taken for chemical analyses. The obtained results showed that the tested soil had an acid reaction ((pH in nKCl 5,01– 4,94), well supplied by humus 3.35–3.96% and poor in available phosphorus (5.12–4.24 mg 100 g-1 soil) and potassium (7.5–3.8 mg 100 g-1 soil). The soil was weakly calcareous, too (the total content of carbonate being 2.4–2.44%).

Table 1. Mean monthly air temperature (°C) and monthly rainfall during the growing seasons of experimental years and long term average (1961-1990)

Year	Months										Sum
	X	XI	XII	I	II	III	IV	V	VI	VII	
	Monthly rainfall (mm)										
2010-11	65.3	130.7	147.3	36.0	76.0	30.9	45.6	120.8	33.2	78.8	**764.8**
2011-12	36.2	7.4	54.6	78.7	182.7	56.7	47.6	46.2	34.2	7.7	**552.0**
1961-90	80	115	91	87	68	60	70	76	72	64	**783**
	Average monthly temperatures (°C)										**Average**
2010-11	10.1	8.5	2.0	-0.6	0.9	6.0	10.5	14.5	18.9	21.2	**9.2**
2011-12	9.3	3.2	2.2	-1.7	-3.5	5.9	10.8	15.0	20.7	24.6	**8.7**
1961-90	9.4	4.7	0.2	-1.3	0.7	4.9	9.0	13.3	16.3	17.9	**7.5**

Cumulative rainfall was 764.8 mm in the first year and 552.0 mm in the second year at the time of the growing season. In contrast to the first year of the study, when the amount of precipitation during October, November and December was 343.1 mm, in the same period during the second year there was significantly less precipitation (98.24 mm). If we take into account the heavy rainfall in February, as well as the long retention of the snow cover that slowed down the vegetation in the second year of research, the first year can be assessed as more favorable for the growth, development and fruiting of triticale. Measured air

temperature values showed that significantly lower air temperatures in November of the second year of the study caused slowed germination and initial growth of plants. Also, significantly lower temperatures were recorded in February 2012 compared to 2011. The higher amount of precipitation in March, April and May 2011 compared to the same in 2012 had a positive impact on the growth and development of triticale in the second part of the growing season. High air temperatures in July 2012 led to a shortening of the grain filling period and accelerated ripening, which had negative consequences on the yield.

Results and Discussion

The 1000 grain mass is an indicator of grain size and represents the relationship between weight and number of grains. The average values of 1000 grain mass in the examined varieties of winter triticale in the two growing seasons are shown in Table 2.

Table 2. 1000 grain mass of winter triticale varieties (g)

Cultivar (A)	1000 grain mass (g)					
	Year					
	2010/11			2011/12		
	Quantity of seeds (B)					
	300 kg ha^{-1}	360 kg ha^{-1}	Average	300 kg ha^{-1}	360 kg ha^{-1}	Average
Odisej	42.2	39.8	41.0	45.6	43.2	44.4
Kg-20	31.8	31.0	31.4	35.2	34.4	34.8
Trijumf	43.8	43.6	43.7	44.9	44.2	44.5
Rtanj	49.0	48.0	48.5	48.1	47.9	48.0
Tango	46.7	45.3	46.0	49.7	49.1	49.4
Average B	42.7	41.5	42.1	44.7	43.8	44.2
LSD	A	B	AxB	A	B	AxB
0.05	0.483	0.305	0.682	0.477	0.301	0.674
0.01	0.661	0.418	0.935	0.653	0.413	0.923

The average of 1000 grain mass for all varieties and both sowing norms in the first year of research was 42.1 g, while in the second year a higher value of this trait was recorded (44.2 g). The obtained average values of the mentioned trait in our study in the first year of the research were at the level of the values reached by Madić et al. (2018), while in the second year of the research the achieved values were higher. All varieties achieved significantly higher values of 1000 grain mass when applying a lower sowing norm compared to the values obtained when applying a higher sowing norm, which is in accordance with the statements of Mirić et al. (2007) who state the rule that increasing the sowing rate gives more uniform and finer seeds and thus less 1000 grain mass. Mut et al. (2005), Baloch et al. (2010) and Laghari et al. (2011) also confirmed that the increase of the sowing norm and the decrease of the vegetation space affect the decrease of the 1000 grain mass. Compared to other cultivars, the cultivar Kg-20 had a significantly lower average value of 1000 grain mass in both years of research, 31.4 g in the first and 34.8 g in the second year of research.

The highest values of 1000 grain mass were recorded in the varieties Rtanj in the first (48.5 g) and Tango in the second year of research (49.4 g). The average values show that both mentioned varieties had significantly higher values of 1000 grain mass in relation to the other tested varieties. Our results confirmed the earlier statements of Protić et al. (2012) who pointed out that 1000 grain mass depends on the variety and ranges widely.

Hectolitre weight as a significant indicator of the technological value of seeds is a complex trait controlled by a large number of genes. The data shown in Table 3 show that the hectoliter weight of the grain differed significantly depending on the tested genotype, but also on the applied sowing norm. Accordingly, the highest average value of hectoliter weight in the first year of research was recorded in the cultivar Trijumf and it was statistically significantly

higher in relation to the hectoliter weight achieved in the cultivars Kg-20 and Rtanj. In the second year of the research, the Odyssey variety had the highest average value of hectoliter weight (69.4 kg). There was no statistically significant difference in terms of hectoliter weight among the Odyssey, Tango and Triumph varieties. On the other hand, the cultivars Kg-20 and Rtanj achieved significantly much lower hectoliter weight compared to the cultivar Odyssey. Average values show that in both years of research, the Kg-20 variety had the lowest hectoliter weight. In both years of research, by applying a higher sowing norm, significantly higher values of hectolitre weight were obtained. A similar tendency of increasing the value of hectoliter mass due to the increase of the sowing norm was obtained by Protić et al. (1988) and Varga et al. (2000).

Table 3. Hectoliter weight of winter triticale varieties (kg)

Cultivar (A)	Hectoliter weight (kg)					
	Year					
	2010/11			2011/12		
	Quantity of seeds (B)					
	300 kg ha^{-1}	360 kg ha^{-1}	Average	300 kg ha^{-1}	360 kg ha^{-1}	Average
Odisej	65.0	71.8	68.4	68.9	69.9	69.4
Kg-20	65.2	67.4	66.3	58.4	65.2	61.8
Trijumf	69.7	71.5	70.6	67.5	70.4	68.9
Rtanj	65.5	68.2	66.8	59.7	68.6	64.1
Tango	65.9	70.8	68.3	66.0	69.3	67.6
Average B	66.3	69.9	68.1	64.1	68.7	66.4
LSD	A	B	AxB	A	B	AxB
0.05	2.133	1.349	3.017	1.797	1.136	2.541
0.01	2.922	1.848	4.132	2.461	1.556	3.480

Grain yield is the most important indicator of plant productivity, so in accordance with that, the conclusion is that the yields achieved have the greatest practical value for each variety. Grain yield is the surest indicator of the existence of differences in productivity between varieties because it represents the end result of the action of both external factors on the plant and the influence of biorhythmic activity of certain physiological and biochemical processes.

Table 4. Grain yield of winter triticale varieties (t ha^{-1})

Cultivar (A)	Grain yield (t ha^{-1})					
	Year					
	2010/11			2011/12		
	Quantity of seeds					
	300 kg ha^{-1}	360 kg ha^{-1}	Average	300 kg ha^{-1}	360 kg ha^{-1}	Average
Odisej	3.49	4.31	3.90	2.84	4.03	3.43
Kg-20	3.28	3.98	3.63	2.95	3.54	3.24
Trijumf	4.19	4.75	4.47	3.46	4.08	3.77
Rtanj	4.23	4.91	4.57	3.57	4.19	3.88
Tango	4.28	5.14	4.71	3.41	4.32	3.86
Average B	3.89	4.62	4.25	3.24	4.03	3.64
LSD	A	B	AxB	A	B	AxB
0.05	0.751	0.475	1.062	0.620	0.392	0.877
0.01	1.028	0.650	1.455	0.849	0.537	1.201

The data in Table 4 show the existence of differences in the achieved grain yields both between the varieties and between the applied sowing norms and the years in which the research was performed. On average, in both years, a higher grain yield of winter triticale was obtained when using a higher sowing norm. In the first year of testing, the Tango variety had the highest grain yield when applying both sowing norms. The highest grain yield in the second year of research when applying the lower sowing norm had the variety Rtanj, and

when applying the higher sowing norm the variety Tango. The highest two-year average yield was achieved by the Tango variety (4.28 t ha^{-1}). The variety that achieved the lowest average yield in both years of research was the variety Kg-20. Analyzing 2011/12, the yields were lower compared to the previous year of the research. Analysis of variance showed that all cultivars in both years of research with the application of a higher sowing norm achieved statistically highly significant higher grain yields compared to the yield achieved with a lower sowing norm. Otterson et al. (2008), Valerio et al. (2009) and Đurić et al. (2010) pointed out earlier that with the increase of quantity of seeds or crop density, grain yield increased substantially in all varieties. The values of average yields show that a higher grain yield was achieved in the first year of research, which according to climatic conditions proved to be more favorable for the cultivation of winter triticale. Higher grain yields achieved in the first year of research can, among other things, be associated with a higher amount of precipitation during the second part of the growing season and their favorable effect on growth, development and fruiting. Similar results were obtained by Ivanova and Kirchev (2014) and Đurić et al. (2015).

Conclusions

Using the results of this research of the effect of variety and quantity of seeds on fertility and fertility parameters of winter triticale, the following conclusions can be drawn:

- On average, for all cultivars and both applied quantities of seeds, the highest yield was obtained with the Tango variety, while the lowest yield in both test years and the application of both quantities of seeds was recorded with the Kg-20 variety.
- The average values of measured hectolitre weight in the tested triticale varieties were higher when applying a higher quantity of seeds.
- The application of a smaller quantity of seeds led to a significant increase in the 1000 grain mass in all cultivated varieties compared to the application of a higher quantity of seeds and it was also higher in the second study year
- All tested varieties achieved higher grain yield in the first year of research and with using a higher sowing norm

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FRUIT QUALITY OF PLUM (*PRUNUS DOMESTICA* L.) CULTIVARS 'ČAČANSKA LEPOTICA' AND 'EMPRESS' AFTER COLD STORAGE

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Abstract

In order to respond to consumers' requirements, it is necessary to supply the market with high quality plum fruit. As the quality of the fruit starts to change immediately after harvest, cold storage is applied in order to maintain fruit characteristics, determined mainly in orchards. Since fruit quality and storage potential depend on cultivar, the aim of this research was to analyze and compare quality-related properties of 'Čačanska Lepotica' and 'Empress' before and after cold storage. Although examined cultivars have different ripening time, they are primarily grown for fresh consumption. Thus, all analyses were performed at harvest and after 28 days of cold storage (0–2 °C, 90–95% relative humidity), followed by a shelf life of three days at 20 °C. Fruit firmness, weight loss, susceptibility to *Monilinia* spp., soluble solids content, titratable acidity, antioxidant capacity, the concentrations of total phenolics, flavonoids and anthocyanins were monitored. After cold storage and subsequent shelf life, an increase in soluble solids content, antioxidant capacity, total phenolics and flavonoids were observed in both cultivars. Significant differences between fresh and stored plums were found in terms of fruit firmness. Fruit of 'Čačanska Lepotica' had higher content of soluble solids and anthocyanins and it was firmer in both analyses' dates compared to fruit of 'Empress'. However, its fruit was more susceptible to *Monilinia* spp. and had significantly higher weight loss than 'Empress' fruit.

Keywords: *Storage, Fruit firmness, Weight loss, Bioactive compounds.*

Introduction

Plum, as a highly perishable stone fruit, has limited shelf life, which represents a serious restraint for its adequate storage and transportation. Due to rapid ripening and softening, plum is usually marketed within a short period of time. However, meeting consumers' demands means supplying the market with high quality plum fruits for at least three weeks after harvest (Vangdal et al., 2007). Nevertheless, growers attempt to store plums in order to achieve better prices later in the season. Therefore, knowledge about fruit's behaviour during and after cold storage is of great importance for maintaining plum quality.

Antioxidants are molecules, which in low concentrations significantly inhibit the oxidation of the substrate (Halliwel et al., 1995). In order to protect biomolecules and tissue, antioxidants react with potentially dangerous free radicals. During this reaction, antioxidants are being oxidized, free radicals are scavenged and the oxidation process is hindered. Hence, the antioxidant capacity is one of the essential determinants of food biological value. Since the antioxidant capacity is largely dependent on the content of total phenolics, flavonoids and anthocyanins (Vinson et al., 2001), the present research evaluated the changes in the content of aforementioned compounds in fresh and stored plums. Although several studies have examined major indicators of plum quality (soluble solids content, titratable acidity, fruit firmness, weight loss) during storage (Mihalache Arion et al., 2014; Vangdal et al., 2007),

changes in bioactive compounds under unfavourable market's conditions (high temperature and low relative humidity) are still indefinite.
Cultivars and rootstocks play an essential role in determination of postharvest storage potential and quality of stone fruits (Crisosto and Costa, 2008; Crisosto et al., 1995). Thus, the aim of this study was to analyze and compare quality-related traits of two plum cultivars with different ripening time, 'Čačanska Lepotica' and 'Empress', before and after cold storage.

Material and Methods

Fruits of two plum cultivars 'Čačanska Lepotica' and 'Empress' (also known as 'Grossa di Felisio') were harvested at the optimal maturity stage for cold storage. Harvest date for 'Čačanska Lepotica' was 26th July 2019, whilst fruits of 'Empress' were picked on 10th September 2019. The fruits were obtained from the commercial orchard, located near Čačak, in which standard agricultural practices were applied. After transportation to the laboratory of Fruit Research Institute, Čačak, fruits were divided into two groups. One group of fruits was used for determination of fruit quality on the harvest date, whilst another group was stored for 28 days in a cold chamber at 0–2 °C and 90–95% relative humidity. In order to simulate market's condition, after removal from the cold chamber, fruits were kept at 20 °C for three days. Therefore, all analyses were performed in two terms, at harvest and after 28 days of cold storage followed by a shelf life of three days.
Twenty fruits of each cultivar were selected for the weight loss test (Korićanac et al., 2020). On every analyses' date ten randomly chosen plums per cultivar were used for determination of fruit's firmness by a hand penetrometer (model FT 327, T.R. Turoni, Forly, Italy); two measurements were made on opposite sides of the fruits and then averaged in order to obtain the mean value expressed in kg/cm^2. Soluble solids content (SSC) was measured on a digital refractometer (model MA871, Milwaukee Instruments, Rocky Mount, NC, USA) and expressed in %. Titratable acidity (TA) was determined by neutralization with 0.1 N NaOH to pH 8.2, using phenolphthalein as indicator. The results were expressed as a percentage (%) of malic acid. Measurements of pH of fruits' pulp were performed by a potentiometer (pH meter Mettler Toledo EL 20-Basic, Schwerzenbach, Switzerland). After removal of samples from cold storage and subsequent shelf life, the number of fruits infected by *Monilinia* spp. was registered.
Antioxidant capacity was determined by two methods, DPPH and ABTS assays. DPPH procedure was conducted according to the method described by Sanchez-Moreno et al. (1998), whilst the ABTS•+ radical cation scavenging activity was determined according to Re et al. (1999). For both aforementioned assays Trolox was used as a standard and the antioxidant capacity was expressed as millimole Trolox equivalent per 100 g fresh weight (mmol TE/100 g fw). Folin-Ciocalteu colorimetric procedure (Singleton et al., 1999) was used for evaluation of total phenolic content (TPC) and the results were shown as milligrams of gallic acid in 100 g fresh sample (mg GAE/100 g fw). Total flavonoids (TFC) were estimated by the aluminium chloride method (Zhishen et al., 1999). Results were expressed as milligrams of rutin equivalent (RE) per 100 g fresh weight. Anthocyanin quantification (TAC) was performed by the pH-differential method (Hosseinian et al., 2008); results were expressed as milligrams of cyanidin-3-glucoside equivalents (c-3-gE) per 100 g fresh weight. All analyses were conducted in three replicates.
The obtained data were subjected to the one-way analysis of variance (ANOVA, F test). Multiple comparisons of means were performed by the Tukey test ($p = 0.05$) using STATISTICA 7.0 software (Statsoft Inc., Tulsa, OK, USA). All data were reported as mean ± standard deviation.

Results and Discussion

Postharvest quality of plum needs to satisfy consumers' demands in terms of appearance, texture, flavour, nutritional value and safety. One of the major factors limiting storage potential of plums are weight loss and excessive softening. Weight loss results in shrinkage and shrivelling of fruits, which highly affects consumers' acceptance. 'Čačanska Lepotica' had higher weight loss in comparison with 'Empress' (Figure 1), which indicates more intensive transpiration during cold storage and subsequent shelf life in this cultivar. Similar results were reported by Guerra and Casquero (2008), who noticed a significant increase of weight loss (6%) after a three-day shelf life in plum cultivar 'Green Gage'.

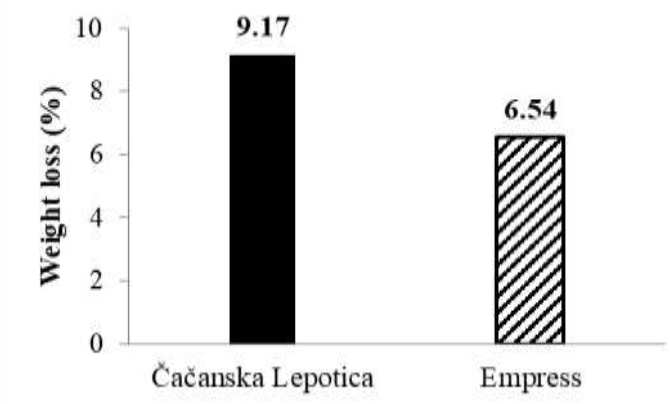


Figure 1. Weight loss in examined plum cultivars after 28 + 3 days of storage

As the result of structural changes of cell wall polysaccharides, particularly pectin solubilisation, fruit firmness rapidly decreased in both cultivars. In 'Čačanska Lepotica' the fruit firmness was reduced by 58.07% after shelf life, whilst 'Empress' had a higher loss of firmness (67.96%). Nevertheless, 'Čačanska Lepotica' had firmer fruit both at the harvest and after storage (Figure 2). Vangdal et al. (2007) reported that the reduction of firmness after two weeks of storage was cultivar dependant and it ranged from 15% to 37%.

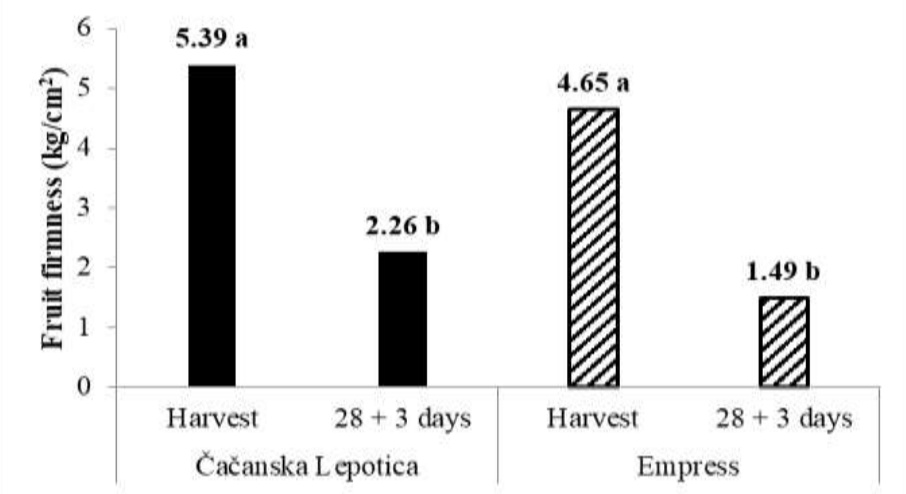


Figure 2. Fruit firmness of examined plum cultivars

Different letters indicate significant differences between analyses dates, according to the Tukey test.

Due to the strong correlation with palatability, SSC and TA are one of the most important quality indicators in plums. An increase of SSC during storage was observed in both examined cultivars (Table 1). On the contrary, TA decreased during the same period, and

consequently higher pH values were detected after storage. Similar findings were obtained by Vangdal et al. (2007) and Guerra and Casquero (2008). Crisosto et al. (2004) reported that plums with SSC ≥ 12% had the maximum consumer acceptance regardless of TA. However, the ratio between the contents of soluble solids and total acids (SSC/TA) is more closely related to the fruit quality than SSC or TA individually (Kader et al., 1982). SSC/TA ratio increased during storage in both cultivars, which is in agreement with the findings of Vangdal et al. (2007). According to the previously mentioned data, fruits of both 'Čačanska Lepotica' and 'Empress' had satisfactory quality-related properties after storage under simulated market's conditions.

Table 1. Soluble solids content (SSC), titratable acidity (TA), SSC/TA ratio and pH value in fruits of examined cultivars at the harvest and after storage

Parameter	'Čačanska Lepotica'			'Empress'		
	ANOVA	Harvest	28 + 3 days	ANOVA	Harvest	28 + 3days
SSC (%)	ns	14.33 ± 1.39	15.08 ± 1.27	***	12.40 ± 1.41 b	15.02 ± 1.98 a
TA (%)	*	1.11 ± 0.03 a	0.90 ± 0.08 b	**	0.97 ± 0.02 a	0.87 ± 0.01 b
SSC/TA	***	12.94 ± 0.26 b	16.76 ± 0.19 a	***	12.78 ± 0.30 b	17.26 ± 0.54 a
pH	***	3.31 ± 0.01 b	3.39 ± 0.01 a	*	3.24 ± 0.03 b	3.33 ± 0.02 a

The presence of different letters in the same row and for the same cultivar indicates that there is significant difference according to the Tukey test. *** statistically significant differences at $p \leq 0.001$; ** statistically significant differences at $p \leq 0.01$; * statistically significant differences at $p \leq 0.05$; ns – non significant difference

Plums are an important source of bioactive compounds, which prevent occurrence of many diseases (Stacewicz-Sapuntzakis et al., 2001) and highly contribute to the antioxidant capacity. High antioxidant capacity, which increased during storage, was observed in both cultivars, regardless of the applied assay (Table 2, Table 3). This is in compliance with the results obtained by Mihalache Arion et al. (2014), who evaluated antioxidant capacity, TPC and TAC of twelve plum cultivars, at the harvest and after storage.

Table 2. Antioxidant capacity evaluated by DPPH and ABTS assays and content of bioactive compounds (total phenolics - TPC, total flavonoids - TFC, and total anthocyanin - TAC) in 'Čačanska Lepotica' fruits

Parameter	'Čačanska Lepotica'		
	ANOVA	Harvest	28 + 3 days
DPPH (mmol TE/100 g fw)	**	1.42 ± 0.03 b	1.55 ± 0.02 a
ABTS (mmol TE/100 g fw)	**	1.30 ± 0.10 b	1.61 ± 0.02 a
TPC (mg GAE/100 g fw)	**	173.10 ± 5.02 b	215.00 ± 7.56 a
TFC (mg RE/100 g fw)	***	134.78 ± 0.92 b	185.67 ± 2.01 a
TAC (mg c-3-gE/100 g fw)	***	46.06 ± 1.03 a	35.35 ± 0.43 b

The presence of different letters in the same row indicates that there is significant difference according to the Tukey test. *** statistically significant differences at $p \leq 0.001$; ** statistically significant differences at $p \leq 0.01$

Although TPC in 'Empress' plums increased by 49.64% after cold storage and subsequent shelf life, it was still significantly lower in comparison with 'Čačanska Lepotica', in which TPC raised by 24.21%. However, storage period did not affect TFC in fruits of 'Empress.'

Table 3. Antioxidant capacity evaluated by DPPH and ABTS assays and content of bioactive compounds (total phenolics - TPC, total flavonoids - TFC, and total anthocyanin - TAC) in 'Empress' fruits

Parameter	'Empress'		
	ANOVA	Harvest	28 + 3 days
DPPH (mmol TE/100 g fw)	**	1.15 ± 0.01 b	1.32 ± 0.04 a
ABTS (mmol TE/100 g fw)	ns	0.97 ± 0.06	1.09 ± 0.07
TPC (mg/100 g fw)	*	96.90 ± 6.75 b	145.00 ± 21.04 a
TFC (mg/100 g fw)	ns	123.45 ± 1.12	124.42 ± 1.10
TAC (mg/100 g fw)	ns	9.46 ± 0.64	9.95 ± 0.12

The presence of different letters in the same row indicates that there is significant difference according to the Tukey test. ** statistically significant differences at $p \leq 0.01$; * statistically significant differences at $p \leq 0.05$; ns – non significant difference

Plum skin colour is an important external characteristic of the fruit, largely dependent on the content of anthocyanins. The obtained TAC in fresh fruits of 'Čačanska Lepotica' was in accordance with the concentrations reported for early plum cultivars (Mihalache Arion et al., 2014), whilst 'Empress' had surprisingly low concentration of these compounds. Interestingly, TAC in 'Čačanska Lepotica' plums decreased during storage, which is contrary to the results reported by Díaz-Mula et al. (2009).

The number of fruits infected by *Monilinia* spp. was determined after 28 days of cold storage followed by a shelf life of three days at 20 °C. 'Čačanska Lepotica' was extremely susceptible to this economically important pathogen with 45% infected fruits. Although Lee and Bostock (2007) reported that the presence of phenolic acids inhibited appressorium formation from germinated conidia and subsequent brown rot lesion development, 'Empress' plums, which contained lower TPC, did not have symptoms of infection in our research. However, it is difficult to compare the cultivars' susceptibility to fungal decay, as the fruits were harvested on different dates, under different climatic conditions. Thus, this result should be considered with caution.

Conclusions

Plum production and marketing require considering several aspects, including the choice of appropriate cultivar, not only in terms of yield but also in relation to fruit quality and storage potential. The present study indicated the difference in changes of quality-related properties during storage in two plum cultivars with different harvesting times. Despite the high susceptibility to *Monilinia* spp., 'Čačanska Lepotica' as an early season cultivar exhibited better postharvest fruit quality (firmer fruit, higher content of soluble solids and bioactive compounds). On the contrary, 'Empress' is a perspective cultivar due to its late harvest time. Nevertheless, it had lower weight loss after shelf life. The obtained results indicate that fruits of both examined cultivars, even after cold storage and shelf life, represent a good source of health-beneficial compounds.

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A SYMBIOTIC BACTERIUM FROM HYBRID ASPEN IN VITRO CULTURE PROMOTES EX VITRO ADAPTATION IN OTHER POPULUS SPECIES

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Abstract

Although plant micropropagation via *in vitro* culture is usually a microbe-free method, some specific plant-associated bacteria can survive disinfection and continue to live in plant tissue culture. The present study reports a potential of such symbionts to promote growth processes even in non-host plant species. The studied bacterium was isolated from the tissue culture of hybrid aspen (*Populus tremuloides* × *P. tremula*) and classified as *Paenibacillus* sp. by 16S rRNA gene analysis. In order to test its effects on a non-host *Populus* species – Berlin poplar (*Populus* × *berolinensis*), this bacterium was either inoculated *in vitro* on the nutrient medium for the otherwise bacterium-free poplar explants or, in the form of freeze-dried powder, mixed into water and applied to the *in vitro*-collected poplar shoots before their adaptation *ex vitro*. Although, in the first case, the initial *in vitro* effect of *Paenibacillus* sp. on poplar explants was negative (lower shoot length and impaired root system development), it still turned to be positive during *ex vitro* adaptation: at the end of the adaptation period, the shoots from the bacterium-treated variant had 71% higher average root length and 118% higher shoot mass than the non-treated control. In the second case, the poplar shoots treated with freeze-dried *Paenibacillus* sp. before the start of *ex vitro* adaptation, finally had 62% higher average root number and 68% higher root mass, in comparison to the control. Thus, the *Paennibacillus* sp, bacterium was confirmed as a helpful tool to obtain stronger poplar plants after the whole micropropagation process.

Keywords: *Paenibacillus, Poplar, In vitro, Micropropagation, Ex vitro adaptation.*

Introduction

Researchers are trying to select or breed trees that not only have better growth, higher stem quality (straight, slightly chamfered, have smaller branches), but are also resistant to diseases, pests, and air pollution. In the context of this task of tree growth improvement, a special interest is recently paid to potentially beneficial interactions between symbiotic bacteria and woody plants. Colonization of endophytic bacteria in the inner tissue of plants can promote plant growth and stress resistance and enhance pathogen control (Hanak *et al.*, 2015). It has been shown that in places where abiotic (non-biological) stressors are present, plants are more dependent on microorganisms that can enhance plant ability to combat stress (Glick *et al.*, 1998). Plant association with microbial endophytes (bacteria or fungi living inside the plants) stimulates growth and nutrition, in addition to increasing plant resistance to stress (Kim *et al.*, 2013). Plant microorganisms can benefit their host plants through a variety of mechanisms, including biological nitrogen fixation (Pankievicz *et al.*, 2015), increased bioavailability of phosphorus (P), iron (Fe), and other mineral nutrients (Bulgarelli *et al.*, 2013), and plant hormone production (De Mota *el al.*, 2008).

The main objective of the present study was the potential of plant growth promotion by the bacterium *Paenibacillus* sp. This specific bacterial strain was isolated from *in vitro* cultures of the hybrid aspen *Populus tremuloides* × *P. tremula* propagated in the laboratory of the Lithuanian Research Centre for Agriculture and Forestry (LRCAF), Institute of Forestry.

Paenibacillus is a genus of optionally anaerobic, endospore-forming bacteria that originally belonged to the genus *Bacillus* and later, in 1993, were reclassified as a separate genus (Ash *et al.*, 1993). Bacteria belonging to this genus have been detected in various environments, such as: soil, water (Preet *et al.*, 2017), rhizosphere, plant material, food, insect larvae, and even glacial ecosystems (Montes *et al.*, 2004).

To test how the *Paenibacillus* sp. bacterium from the LRCAF laboratory affects woody plant species other than its native hybrid aspen, Berlin poplar was taken as a model plant. Berlin poplar (*Populus* × *berolinensis*) belongs to the same genus as aspens but, within this genus, it is still genetically quite distant from aspens. Berlin poplar is a hybrid that, in 1865, originated in the Berlin Botanical Garden after crossing of black poplar (*Populus nigra* L.) with laurel poplar (*Populus laurifolia* Ledeb.) (Rehder, 1934).

Two different approaches were used for the application of *Paenibacillus* sp. bacterium to *in vitro*-propagated Berlin poplar shoots before the start of the adaptation of these shoots to *ex vitro* conditions. In the first case, the bacterium was applied by the spot inoculation of the nutrient medium still during the stage of *in vitro* culture. In the second case, the bacterium, in the form of freeze-dried powder was applied to the shoots already collected from *in vitro* culture and just before their planting *ex vitro*. Both approaches aimed to test if *Paenibacillus* sp. was able to change the morphological development of Berlin poplar and if its application could result in a better quality of *ex vitro*-adapted plants.

Material and Methods

Two distinct experiments were conducted on Berlin poplar (*Populus* × *berolinensis*) clonal shoot cultures. These cultures were established in the laboratory of LRCAF Institute of Forestry (Lithuania) in 2017 and kept *in vitro* for two years via a series of bimonthly subcultures, before being used for the experiments described in this study.

In the first experiment, poplar shoots were cultured on the solid Murashige and Skoog (MS) nutrient medium containing 20 g l^{-1} sucrose and 4 g l^{-1} Gelrite (gellan gum), with the pH value set at 5.8. This medium was also supplemented with 3 μmol l^{-1} of plant hormone abscisic acid (ABA). The *Paenibacillus* sp. bacterium used in the experiment was cultured on the solid Lysogeny broth (LB) medium composed of 10 g l^{-1} tryptone, 5 g l^{-1} yeast extract, and 5 g l^{-1} NaCl. 10-mm-long apical shoot segments cut from the *in vitro*-grown Berlin poplar shoot cultures were used as explants. These were placed in glass jars, 72 mm of height and 68 mm of diameter. Each jar contained 30 ml of MS medium and four vertically inserted poplar explants. A day before the insertion of poplar explants, the MS medium in the jars was spot inoculated with *Paenibacillus* sp. bacterium (four spots in each jar, corresponding to the four sites intended for the later insertion of poplar explants). The bacteria used for the spot inoculation were taken on the tip of a metal needle from the *Paenibacillus* sp. colonies formed on the surface of solid LB medium. In the control variant, poplar explants were planted on a bacterium-free nutrient medium. The explants were cultured *in vitro* for six weeks under a 16-hour white-light photoperiod and 25/18°C (day/night) temperature mode. After six weeks, the morphological parameters of *in vitro*-grown plants were recorded and then the apical shoots, approximately 20 mm long, were harvested, placed in a sterile container with water, and kept overnight at 4ºC. Jiffy-7 peat tablets, 24 mm in diameter, were used for *ex vitro* adaptation of poplar shoots. Prior to planting of shoots, the tablets were soaked in water for 1 hour. Afterwards, the water-swollen tablets with inserted shoots were placed in plastic boxes covered with transparent closures, with each box containing 40 tablets. The *ex vitro*-planted poplar shoots were put under the same light and temperature regime as *in vitro* cultures (described above), and 75% relative humidity was maintained indoors. The *ex vitro* cultures were grown for six weeks until final registration.

In the second experiment, all Berlin poplar explants were first cultured on bacterium-free MS nutrient medium under the same conditions as in the previous experiment, except that ABA was not present in the medium. Then 20 mm long explants (apical shoot segments) were harvested from the *in vitro* culture, placed in a sterile container with water, and kept overnight at 4ºC. For the experiment, the poplar shoots, before being planted to peat tablets (as described above), were treated with lyophilized (freeze-dried) *Paenibacillus* sp. powder. . The powder of lyophilized *Paenibacillus* sp. was mixed with tap water at the concentration of 6 g l^{-1} (mass of bacterial powder per water volume). Berlin poplar shoots harvested from the *in vitro* culture were soaked and kept for 30 min in this aquatic *Paenibacillus* sp. mixture. In the control variant, the poplar shoots were soaked for 30 min in plain tap water. After the soaking, the poplar shoots from both variants (bacterium-treated and control) were planted in peat tablets following the same methodology as described for the *ex vitro* stage of the first experiment. The illumination for the *ex vitro* cultures was provided by red/blue light emitting-diodes (LEDs), arranged in a 5/2 ratio. After six weeks of *ex vitro* culturing, the final registration was performed.

The results presented in this study include the following parameters: shoot length, shoot number, shoot mass, root number, root length (considering the longest root of each shoot), and root mass. For the comparison of the obtained results, a two-tailed Welch's t-test (Welch, 1947) intended to compare samples with possibly unequal variances was performed to calculate the probability that the means of the different variants are equal.

Results and Discussion

The effects that the nutrient medium's inoculation with *Paenibacillus* sp. bacterium had on the *in vitro*-grown Berlin poplar plants are shown in Figure 1. It was found that the bacterium's presence in the nutrient medium had a negative effect on shoot growth (Figure 1A). Explants grown on the bacterium-inoculated medium had, on the average, 31% shorter shoots than explants from the control group. Similar but even more remarkable results were obtained considering root development. Explants formed roots (Figure 1B) much more intensely in the control than in the bacterium-treated variant. Without the bacterium, the average root number per plant was as high as 3.65±0.37 and, under the influence of the bacterium, this number was reduced to only 0.88±0.25 roots per plant. Also, the roots formed by the control explants were longer than in the bacterium-treated variant (Figure 1C). All these results suggest that the *Paenibacillus* sp. bacterium had a negative immediate effect on the *in vitro* development of Berlin poplar explants.

However, a strikingly different picture was obtained considering how the explants, collected from the bacterium-inoculated *in vitro* medium, developed during *ex vitro* adaptation. The results illustrating this *ex vitro* development are shown in Figure 2. The shoots grown *ex vitro* by the bacterium-treated explants were not shorter than in the control variant (Figure 2A). Moreover, it was found that the previous (*in vitro*) exposure to the bacterium resulted in poplar plants that, after *ex vitro* adaptation, had an increased number of shoots (Figure 2B) and an even 53% higher overall shoot mass (Figure 2C), in comparison to the control. After the *ex vitro* stage, a positive effect of the *Paenibacillus* sp. bacterium was also observed considering root development. Particularly, it was found that the longest root developed by each individual plant was, on the average, 41% longer among the bacterium-affected plants than in the control group (Figure 2E). Thus, the final effect of the *in vitro*-applied *Paenibacillus* sp. bacterium on Berlin poplar development, including, particularly, the *ex vitro* stage, can be considered as a positive one.

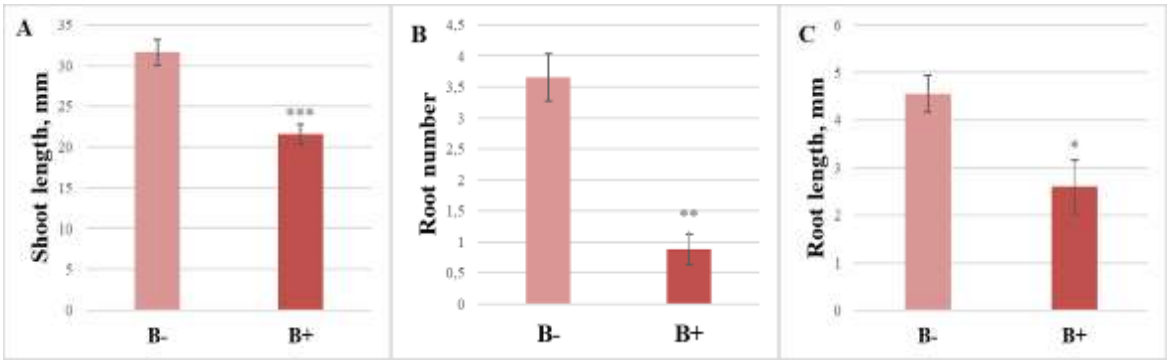


Figure 1. Characterization of Berlin poplar (*Populus* × *berolinensis*) shoot explants after six weeks of *in vitro* culture: A – shoot length; B – root number; C – root length. Different conditions, considering a bacterial treatment, are denoted: "B-" – control; "B+" – medium inoculated with *Paenibacillus* sp. Statistically significant differences between these are indicated: *p < 0.05, **p<0.01, ***p <0.001.

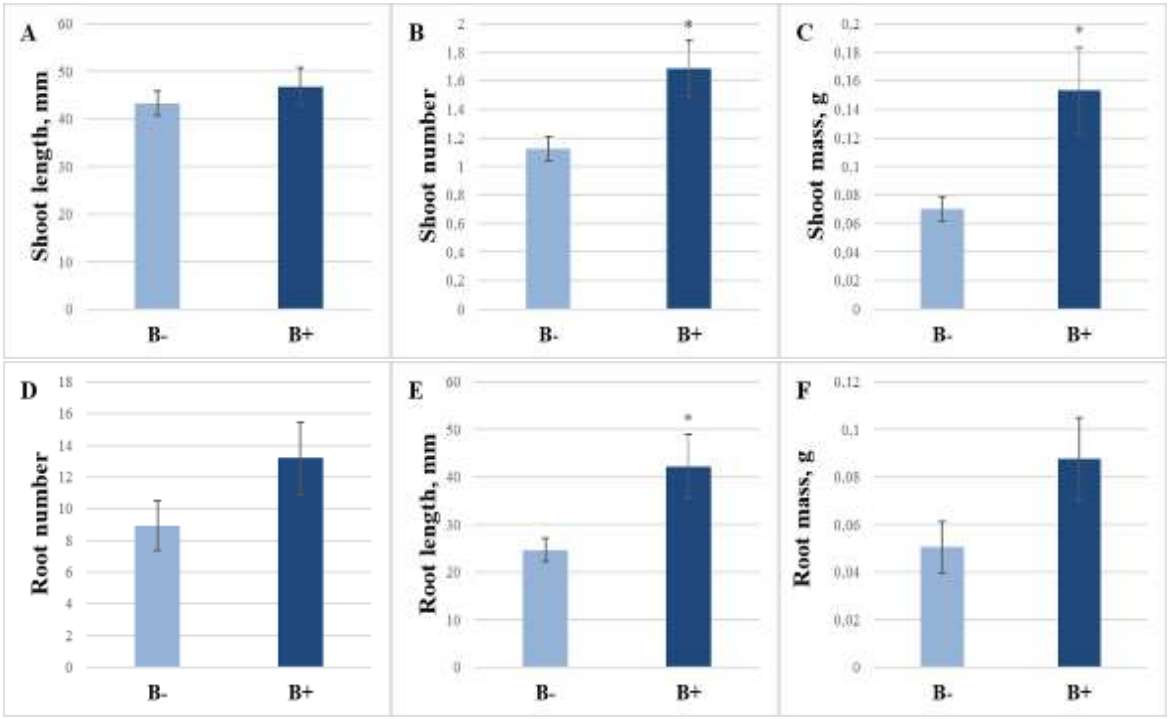


Figure 2. Characterization of Berlin poplar (*Populus* × *berolinensis*) cloned plants after six weeks of *ex vitro* adaptation that followed six weeks under different conditions (considering a bacterial treatment) *in vitro*: A – shoot length; B – shoot number; C – shoot mass; D – root number; E – root length; F – root mass. Different conditions, provided during *in vitro* culture, are denoted: "B-" – control; "B+" – medium infected with *Paenibacillus* bacteria. Statistically significant differences between these are indicated: *p < 0.05.

The results of the experiment in which Berlin poplar shoots were treated with the lyophilized powder of the *Paenibacillus* sp. bacterium are shown in Figure 3. Because this bacterial treatment was conducted just before the planting of shoots under non-sterile conditions, the obtained results illustrate the *ex vitro* development solely. Although no statistically significant changes were induced by the bacterial treatment in shoot development, the effects on root development were clear. The bacterial treatment had a positive effect on root formation, as the

average number of roots formed on the bacterium-affected shoots was 38% higher than in the control group (Figure 3D). Correspondingly, the plants developed from the bacterium-affected shoots had, in comparison to the control group, a 40% higher root mass (Figure 3F).

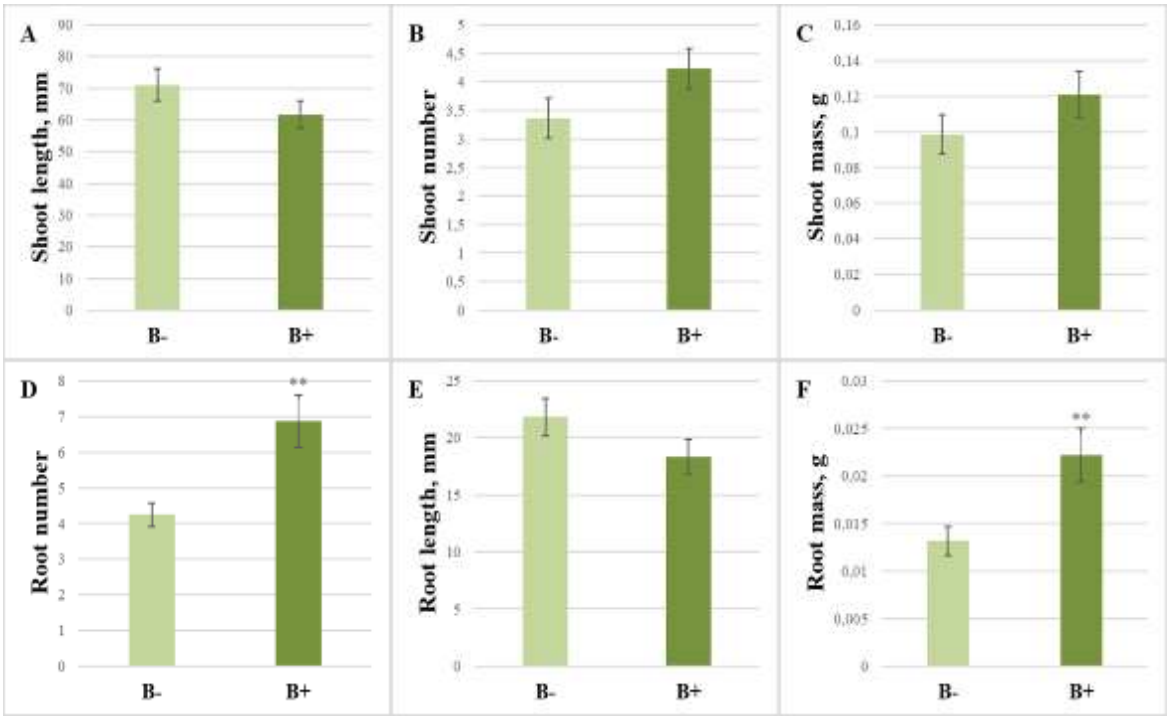


Figure 3. Characterization of Berlin poplar (*Populus* × *berolinensis*) cloned plants after six weeks of *ex vitro* adaptation: A – shoot length; B – shoot number; C – shoot mass; D – root number; E – root length; F – root mass. Different treatments, provided before the planting *ex vitro*, are denoted: "B-" – control (water); "B+" – water mixed with 6 g l^{-1} of freeze-dried *Paenibacillus* sp. Statistically significant differences between these are indicated: **p <0.01.

The obtained results show that, although the *Paenibacillus* sp. bacterium is not helpful for the direct improvement of Berlin poplar development during *in vitro* culture, the clear advantage of bacterium-treated explants can be seen during the subsequent *ex vitro* development. The potential of a *Paenibacillus* sp. bacterium to increase the number of shoots and roots on poplar explants was previously reported by Ulrich *et al.* (2008), although these authors observed this positive bacterial effect during *in vitro* culture and not *ex vitro*, in contrast to the present case. However, the exact comparison of the results is problematic because the methods of bacterial application, as well as *Paenibacillus* and poplar genotypes used in both studies, were different. Still, some basic mechanisms can be suggested to explain the positive effects of *Paenibacillus* bacteria on plants, observed in different studies. Bacteria of the genus *Paenibacillus* are reported as producers of plant hormone auxin (Da Mota *et al.*, 2008). Because this hormone is, among its other functions, responsible for plant cell division, most *Paenibacillus* species are included in the list of plant growth-promoting bacteria (Hanak *et al.*, 2014). From the practical point of view, the present results suggest that, to obtain better shoot growth, the explants can be affected by the bacterium while still being cultured *in vitro*. However, for better root development, it is possible to use lyophilized bacteria powder to affect plants just before the start of an *ex vitro* culture.

Conclusions

The present study showed the potential of the *Paenibacillus* sp. bacterium, isolated from the tissue culture of hybrid aspen (*Populus tremuloides* × *P. tremula*), to promote development of a non-host tree Berlin poplar (*Populus* × *berolinensis*) during its micropropagation process. Although the bacterium's inoculation on plant nutrient medium initially resulted in impaired development of Berlin poplar explants *in vitro*, still, after *ex vitro* adaptation, the shoots from the bacterium-treated variant had 71% higher average root length and 118% higher shoot mass, in comparison to the non-treated control. Another approach of bacterial application, when poplar shoots collected from *in vitro* culture were treated with freeze-dried *Paenibacillus* sp. powder just before the start of *ex vitro* adaptation, resulted in 62% higher average root number and 68% higher root mass in comparison to the control.

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WEED CONTROL ABILITY IN SWEET MAIZE OF SINGLE SOWN LEGUME COVER CROPS COMPARED TO THEIR MIXTURES

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Abstract

To achieve efficient weed control through cover cropping, the plant species chosen is very important. Growing different legume cover crop (CC) species single and in mixtures with grass may increase the number of provided ecosystem services, including reliable suppression of weeds. We conducted an experiment using randomized complete block design with four replications in 2014/15 - 2015/16 growing seasons, at the Experimental Field of Maize Research Institute in Zemun Polje (Serbia). Single cover crops were considered as the main factor (common vetch and field pea), mixtures common vetch and field pea with winter oats and traditional variant, without coverage on biomass. Green biomass of the cover crops was incorporated in the soil, and treatments with favorable establishment and above-average biomass yields tended to suppress weeds by showing lower weed dry matter and weed numbers. The weediness of sweet maize was much higher in the second year of investigation. The number of weed species, fresh and dry biomass of weeds were the least in the variants with mixtures, while the number of plants per specieswas the lowest in the single cover crops. So, mixtures were not as effective as legume single sown CCs, species combinations increased resilience against adverse weather conditions, an advantage to achieving efficient weed control over a long-term period. The statistically significant difference in the fresh biomass of weeds obtained in the control variant (540.46 and 385.88 g) was especially pronounced compared to the variants with single cover crops (391.63 and 486.53 g) and their mixtures (260.00 and 250.78 g), indicating a higher proportion of perennial weed species.

Keywords: *cover crops, plant diversity, main crop, weed management.*

Introduction

One of the major problems in growing broad-leaved crops is weediness, especially in the initial stages of vegetative growth and development. In conventional production, this problem is usually solved by direct weed control measures, i.e. by applying herbicides. In sustainable farming systems, which today's producers need and generally approaching, weed control is mainly based on preventive management practices and corrective herbicide application. In such circumstances, on the one hand, and global climate change, on the other hand, measures of increased land cover during the year, such as crop rotation, cultivation of inter- and cover crops (CC), play a significant role. The integration of cover crops into crop rotations has become a practical strategy by producers (Schappert et al., 2019). Cover crops are normally grown between two main crops to reduce erosion and to improve soil characteristics like nitrogen content, phosphor availability and soil structure (Hartwig and Ammon, 2002), increase efficiency of water usage, improve to weed control (Dolijanović et al., 2012) and they also provide services that reduce pests, and pathogens (Fourie et al., 2016). Some cover crop species growing single or in mixture with grass are able to survive the harsh conditions

over winter and continue to provide this service in early spring and after sowing the main crop they remain on the surface as an undercrop and it is a living mulch. Another strategy is to grow during the fall and winter, and in the spring, cover crops are normally terminated by mechanical or chemical methods before sowing following main crop, and then it is dead mulch. Under both strategies, plant residues continue to release the remaining allelochemicals that are contained in the dead (Tabaglio et al., 2013) or living plant material (Dolijanović et al., 2013). Examining the influence of live and dead mulch on weediness and yield of sweet maize, Dolijanović et al. (2013) states that living mulch in spring-sown cover crops had a positive impact on lower weediness, and opositelly, negative impacts on sweet maize yield. The main crop of sweet maize was not competitive enough with ground cover, mainly because of limited soil moisture and nutrients, especially between the rows of sweet maize being possessed by living mulch. Single sown cover crops (common and hairy vetch, oats and fodder kale) were more successful in weed control than mixtures (common and hairy vetch + oats). Schappert et al. (2019) states that CC mixtures might substantially contribute to the success of biological weed control if the weed suppression mechanisms of different plant species and their ideal composition within mixtures can be identified. The objective of this study was to determine the effect of different winter (dead mulch) and single sown legume cover crops and their mixtures with oats on weed infestation of sweet maize.

Material and methods

The experiment included two single *winter cover crops* (common vetch and field pea), mixtures winter cover crops with oat and traditional production method, classical plowing in the fall and keeping bare soil uncovered during the winter. All of the varieties being used as a cover crops belongs to theInstitute of Field and Vegatble Crop, Novi Sad. Crops were grown under rainfed conditions. Field experiments were conducted in 2014/15 and 2015/16 at Maize Research Institute, Zemun Polje, in the vicinity of Belgrade (44°52'N 20°20'E). The soil was slightly calcareous chernozem with 47% clay and silt, and 53% of sand. The soil properties in layer 0-30-cm of depth were as fallow: 3.22% organic matter, 0.19% total N, 1.9% organic C, 16.2 and 22.4 mg per 100 g soil of available P_2O_5 and extractable K_2O, respectively, 1.38% total $CaCO_3$ and pH 7.3. The experiments were located in different plots in each year and winter wheat was the precceding crop. Fallowing nitrogen fixation rates in legume crops, as well recommended fertilization, we came up to the required amount of macronutrients for sweet maize (120 kg ha^{-1} N, 90 kg ha^{-1} P_2O_5 and 60 kg ha^{-1} K_2O). In the fall period, before planting of cover crops we have entered the entire amount of P and K in the forms of monopotassium phosphate plus additional quantity of nitrogen 50 kg ha^{-1} by ammonium nitrate, and on the two control variants, also all of P_2O_5 i K_2O and 40 kg ha^{-1} N in the form AN. In the next spring (May 21 2015 and April 28 2016) leguminous cover crops had received another 30 kg ha^{-1} N in the form of AN (remaining 40 kg ha^{-1} considered to be provided by nitrogen fixation), and control plots another 80 kg ha^{-1} N, also in the form of AN. The experimental plots being ploughed in the autumn, have followed one pass of a disk harrow and a field cultivator prior to sowing. The entire quantityof nitrogen, phosphorousand potassiumforspring cover cropswere applied just prior to planting, with soil preparation.
Sowing of cover crops were done manually in November 13, 2014 and November 04, 2015.Mowing the above-ground biomass of winter cover crops were performed 7-10 days before planting of sweetmaize. Sowing of sweet maize were done on May 21 in 2015, and April 28 in 2016 year. The estimation of weed infestation in sweet maize was conducted in early July for both years. Crops were harvested 22-24 days after pollination. harvest was performed on August 21, 2015 and August 03, in 2016.

Experimental design

The experiment was in factorial setting with two factors in RCBD with four replications. Sweet maize was sown in density of 65.000 plants ha^{-1}. The inter-row distance was 70 cm, while within-row plant distance was 22 cm. The Zemun Polje (ZP) sweet maize hybrids ZP 424su (FAO maturity group 400) was sown. The basic plot size was 16.8 m^2 (2.8 m by 6.0 m).

Measurements and statistical analysis

Total numberweed species, the number of plants per species, fresh and dry biomass of weeds in sweet maize crops were analysed in this study. All stated parameters in weeds were determined from samples taken from 1m^2. The weed infestation analysis was performed at beginning of July. Following weed sampling, manual hoeing was done in order to suppress weeds pressure in sweet maize. The yield data were underwent to ANOVA for the factorial trials set up according to the plan for two factors, where means differences were tested by the least significant difference (LSD) test (Gomez and Gomez, 1984). The meteorological conditions during the maize growing season are presented in Table 1.

Table 1. Mean monthly air temperatures and precipitation sums from April to August at ZemunPolje

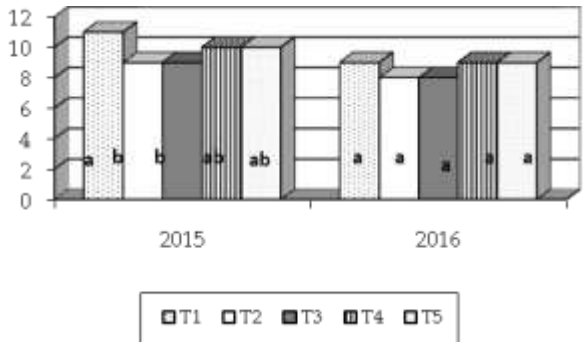
Months	Temperature (°C)		Precipitation (mm)	
	2015	2016	2015	2016
April	13.5	15.5	30.7	53.9
May	19.1	17.5	80.7	71.3
June	21.9	22.5	38.6	152.2
July	26.8	24.4	10.6	35.0
August	26.0	22.3	49.5	60.8
Average/Sum	21.5	20.4	210.1	373.2

The first year of the research is characterized by a higher average air temperature and a significantly lower amount of precipitation (Table 1). In such a situation, only the number of weed species in sweet maize increased, while the number of plants per species, fresh and dry biomass, was significantly higher in 2016. Therefore, the growing of cover crops is completely justified in drier years, which have become more frequent in the conditions of climate change in recent years.

Results and discussion

The number of weed species

In all cover crop treatments and control, a larger number of species was observed in the first year of investigating. A statistically significant difference in the number of species was observed only between T1 (common vetch) and T2 and T3 (field pea and a mixture of common vetch and oats) (graph 1). A larger number of weed species in mixtures in relation to single cover crops was observed in previous papers (Dolijanović et al., 2015; Finney et al., 2016; Baraibar et al., 2018).

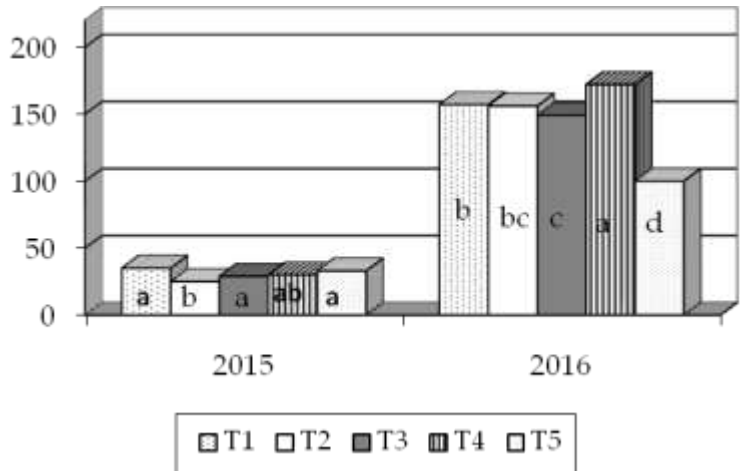


Graph. 1. The number of weedsin sweet maize after cover crops in 2015 and 2016year
Different letters indicate significant difference between treatments at $p < 0.05$; LSD test

Total number of plants per species

Table2.Total number of plants per species ($N^o m^{-2}$) in sweet maize growing after cover crops

Weed species 2015	T1	T2	T3	T4	T5	Average
Chenopodium album L.	13	7	5	9	9	8.6
*Datura stramonium*L.	5	4	2	6	4	4.2
Amaranthusretroflexus L.	3	3	4	1	1	2.4
Amaranthushybridus L.	3	4	3	5	4	3.8
Amaranthusalbus L.	2	3	4	2	4	3.0
Sorghum halepense (Pers.) L.	2		3	1	1	1.4
Solanum nigrum L.	2	1	6	3	3	3.0
Chenopodiumhybridum L.	2		1	1		0.8
Bilderdykia convolvulus L.	1	1	1	1	3	1.4
*Ambrosia artemisiifolia*L.	1					0.2
Panicum crus-galli	1				1	0.4
*Convolvulus arvensis*L.		1				0.2
Portulacaoleracea		1		1	3	1.0
Total	35	25	29	30	33	30.4
		2016				
Solanum nigrum L.	98	97	98	89	57	87.8
Bilderdykia convolvulus L.	8	4		7	12	6.2
Chenopodium album L.	4	1	6	4	2	3.4
Sorghum halepense (Pers.) L.	18	21	1	2	3	9.0
Amaranthusretroflexus L.	9	14	8	25	8	12.8
Chenopodiumhybridum L.	1		6		1	1.6
*Datura stramonium*L.	1		3	4	3	2.2
Amaranthushybridus L.	5	7	11	26	6	11.0
Amaranthusalbus L.	13	12		6	8	7.8
*Amaranthusblitoides*L.			16			3.2
Portulacaoleracea				9		9.0
Total	157	156	149	172	100	146.8



Graph.2. Total number of plants per species ($N^o m^{-2}$) in sweet maize after cover crops
Different letters indicate significant difference between treatments at $p < 0.05$; LSD test.

Dominant weed species are characteristic of the southeastern Srem locality and sweet maize. In contrast to the number of species, the number of weeds, a statistically significantly smaller number was observed in 2015. (table 2; graph 2). The advantage of treatment with cover crops is more pronounced in a climatologically less favorable year of investigating. In the second year, single sown legume cover crops showed an advantage over the mixtures and the control variant.

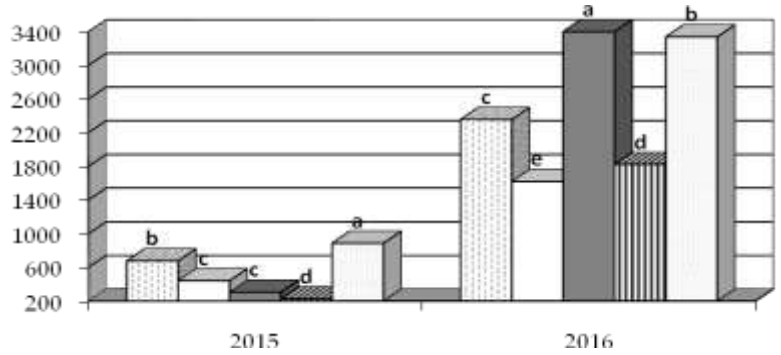
Fresh biomass of weeds

Significantly higher fresh mass of weeds was measured in 2016, which indicates a higher presence of broadleaf weeds in conditions of increased precipitation and lower air temperatures. The highest total fresh weight was 886.4 g m^{-2} (conventional system) in 2015 and 3892.0 g m^{-2} in 2016 in mixture common vetch and oats and the lowest in single field pea and mixture field pea and oat (table 3).In addition to the influence of the cover crop, the rapid growth of the main crop after sowing and the increase of competitive ability also affected the reduction of weeds (Dorn et al., 2015). Good choice of mixture CC species with physical and chemical weed suppression mechanisms may increase the weed control success (Schappert et al., 2019).

Table3.Fresh biomass of weeds (g m^{-2}) in sweet maize after cover crops

Weed species 2015	T1	T2	T3	T4	T5	Average
Chenopodium album L.	414.4	319.1	47.2	91.6	425.6	259.6
*Datura stramonium*L.	41.1	31.1	6.1	30.2	43.8	30.5
Amaranthu sretroflexus L.	117.2	13.5	61.0	5.2	16.3	42.6
Amaranthus hybridus L.	12.1	24.1	27.6	21.5	101.9	37.4
Amaranthus albus L.	8.3	10.3	26.5	12.7	29.3	17.4
Sorghum halepense (Pers.) L.	11.7		41.6	7.1	14.8	15.0
Solanum nigrum L.	17.4	7.7	25.4	18.4	17.1	17.2
Chenopodium hybridum L.	24.0		9.1	1.0		6.8
Bilderdykia convolvulus L.	8.5	17.3	60.9	30.8	157.2	54.9
Ambrosia artemisiifolia L.	15.2					3.0
Panicum crus-galli	7.0				28.5	7.1

Convolvulus arvensis L.		10.2				2.0
Portulaca oleracea		7.1		7.8	51.9	13.4
Total	676.9	440.4	305.4	226.3	886.4	507.1
		2016				
Solanum nigrum L.	1065.1	723.0	1982.5	474.8	1597.5	1168.6
Bilderdykia convolvulus L.	31.4	10.1		47.0	369.7	91.6
Chenopodium album L.	124.0	24.2	243.0	125.9	49.5	113.3
Sorghum halepense (Pers.) L.	199.5	350.8	24.5	40.5	34.7	130.0
Amaranthu sretroflexus L.	464.8	467.8	535.4	602.8	756.2	565.4
Chenopodium hybridum L.	7.6		202.4		22.3	46.5
*Datura stramonium*L.	20.9		100.5	15.9	69.9	41.4
Amaranthus hybridus L.	139.4		561.4	419.6	225.7	269.2
Amaranthus albus L.	303.4	44.8		60.6	211.8	124.1
Amaranthus blitoides L.			242.3			48.5
Portulaca oleracea				36.6		7.3
Total	2356.1	1620.7	3892.0	1823.7	3337.3	2606.0



Graph.3. Fresh biomass of weeds (g m^{-2}) in sweet maize after cover crops
Different letters indicate significant difference between sites at $p < 0.05$; LSD test.

Air dried biomass (g m^{-2})

Results of air dried above-ground biomass of cover crops are presented in table 4.The highest air dried biomass of weeds in both years of investigating was measured in the control variant, and the lowest mainly in mixtures of cover crops with oats (first year) and in single cover crops of winter field pea and mixture of pea with oats (second year). Air dried biomass of weeds is an indirect indicator of the presence of broadleaf (annual and perennial) weeds. In organic cereal cropping systems, with or without a cover-crop, perennial weeds such as *C. arvense*, *Sonchusarvensis* and *E. repens* are of great concern in many temperate countries (Melander et al., 2012). Researchers and farmers claim that perennial creeping weeds threaten the future of organic cereal production, unless the management of these weeds is given due consideration in crop rotation (Sundheim et al., 2014).

Table 4.Air dried biomass of weeds (g m^{-2}) in sweet maize after cover crops

	T1	T2	T3	T4	T5	Average
2015	169.6^{b}	126.5^{c}	71.2^{d}	60.0^{d}	259.2^{a}	137.3
2016	436.7^{c}	409.7^{c}	651.9^{a}	350.9^{d}	569.7^{b}	483.8
Average	303.2	268.1	361.6	205.5	414.5	310.6

Different letters indicate significant difference between sites at $p < 0.05$; LSD test.

Conclusion

Favorable weather conditions during the second year of investigation have resulted in an increasing weed infestation of main crop. Among all variants with winter cover crops, plot weediness of main crop was lower comparing to control variants in both years of investigation. In the first year of investigating, the number of weeds and number of plants per species was higher in the mixtures compared to single cover crops, while the fresh and air-dry mass of weeds was higher in single cover crops. In the second year of the study, the opposite trend was observed. In addition, cost inputs were reduced, but no other common benefits in the long term were found on winter cover crops and their mixtures (increase of organic matter, increase of biodiversity, conservation of water, N, etc.).

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THE SYLLEPTIC SHOOTS OF APRICOT (*PRUNUS ARMENIACA* L.) - FLOWERING PHENOPHASE AND PROPERTIES OF FRUITS

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Abstract

The growth of young shoots of apricot during the growing season takes place through two or three stages of growth. During the vegetation sylleptic shoots can develop. Their number and length are condicioned by the growing conditions, cultivar and applied growing technology. During 2018 impact of heading cut of shoots within early summer apricot pruning on sylleptic shoots development was investigated. The research included three cultivars ('Goldrich', 'Roxana', and 'Hungarian Best') grafted on Myrobalan seedlings (*Prunus cerasifera* Ehrh.). The cutting back of shoots was done in time of their intensive growth in the first half of vegetation (10th of June). In the following 2019, the reaction to pruning was studied by determining the number and length of developed sylleptic shoots. Also, flowering time and the most important properties of fruits on sylleptic shoots in relation to other types of bearing wood in the same canopy were investigated. The results showed that the summer apricot pruning resulted in the development of sylleptic shoots in all investigated cultivars. The number of sylleptic shoots per one-year old shoot varied from 1.90 ('Hungarian Best') to 4.05 ('Roxana'), which is a significantly higher number than when they developed spontaneously (0.10 on average). The average length of spontaneously developed sylleptic shoots was 14.60 cm, while the average length of sylleptic shoots developed after heading cut was 19.17 cm. Flowering phenophase on sylleptic shoots in relation to other types of bearing wood in the same canopy was delayed 2-3 days and lasted one day longer. The weight of fruits developed on sylleptic shoots ranged between 55.24 g ('Hungarian Best') and 62.35 g ('Goldrich') and was on average 11.30% lower in comparison with weight of fruits developed on other types of bearing wood in the same canopy.

Key words: *apricot, heading cut, start and duration of flowering, fruit weight.*

Introduction

Apricot is a fruit species that is adapted to the cold winter without major changes in temperature, short spring, after which comes the dry continental summer. It is very sensitive to temperature oscillations during winter and early spring, when flower buds and flowers freeze very often. One of the ways to prevent freezing of flowers in the spring can be the extended duration of the flowering phenophase. Prolonged flowering in apricots is mainly conditioned by climatic conditions (Glišić *et al.*, 2017), but also may be a consequence of the fact that the flowers on sylleptic shoots open later and lead to a longer duration of flowering in such trees (Milatović, 2013). Sylleptic shoots can developed spontaneously, but their formation can be induced by summer pruning, too. Summer pruning of apricots has long been applied in European countries with developed apricot production (Krška *et al.*, 2012). Šit (1958) was among the first authors who points out the importance of summer pruning of apricot. He noticed that the cutting of the young shoots at the end of May and in the first half of June causes the appearance of sylleptic shoots wich bring flower buds that bloom much later than those where the young shoots are not pruned. Previous experiments have also shown that summer pruning has a positive effect on the formation of flower buds, improves

fruit quality, controls the development of trees, etc. (Küden and Kaska, 1995; Demirtas *et al.*, 2010). In general, summer pruning can be applied before and after harvest, taking into account the development of flower buds. If the pruning of the young shoots is done earlier, before the harvest, sylleptic shoots usually develop from the summer buds, and flower buds are formed on them (Šit, 1958; Demirtas *et al.*, 2010). Also, summer pruning has a very positive effect on the reduction of tree vigour, earlier fruiting and fruit quality (Son and Küden, 2002; Szklarz *et al.*, 2011). However, in apricot orchards in Serbia, summer pruning is being applied only recently, and it is usually made from the end of harvest until the last decade of August (Milošević *et al.*, 2008; Jovanović *et al.*, 2010). The need for the application of summer apricot pruning has been further increased by the fact that in our orchards, the dominant rootstock is the Myrobalan seedling, which is very vigourous. Pruning before harvest, which involves mainly procedures on the young shoots and has an inhibitory effect on their growth, is practically not applied or is applied to a very small extent. In addition, the terms in which this pruning should be applied are not precisely defined. The aim of this paper is to analyze the influence of cutting of young shoots of apricots on the development of sylleptic shoots. Also, the goal is to determine the differences in the phenophase of flowering and some traits of fruits developed on sylleptic shoots compared with other types of bearing wood.

Materials and methods

The investigation was conducted in apricot experimental orchard located in the village Gornja Gorevnica near Čačak during 2018 and 2019. The research included three cultivars ('Goldrich', 'Roxana' and 'Hungarian Best') grafted on Myrobalan seedlings (*Prunus cerasifera* Ehrh.). Planting distance is 6.0 m × 3.5 m (480 trees ha^{-1}). Training sistem is vase with three to four scaffold branches.

Vase training sistem is characterised by tendency to bare scaffold brunches and moving flowering buds to the periphery of the crown, so from the second year after planting, summer pruning was applied. As one of summer pruning measures, the cutting of young shoots on four to six buds was applied. The cutting of young shoots was applied in time of intensive growth of shoots in first part of vegetation (10^{th} June). In total 90 shoots in three replication were cutted of, and after that, development of sylleptic shoots was observed. Trees without cutting of shoots were used as a control.

Next year, at the end of March, the reaction to the cutting of the young shoots during the previous vegetation (number and length of developed sylleptic shoots) was recorded. Also, the beginning and course of flowering on sylleptic shoots were recorded. Finally, fruit weight, stone weight and flesh percentage of fruits developed on sylleptic shoots were measured and compared with fruits developed on other types of bearing wood and trees on which young shoots were not pruned.

The data were statistically processed by calculating the mean value, and its absolute variability was defined using the standard mean error (±SE). Statistical analysis and testing of the significance of the obtained differences were performed by analysis of variance and LSD test for the significance level of $P \leq 0.05$ using the statistical program ANOVA (SPS Statistica, Software 5.0).

Results and discussion

The adaptation of apricots to typical continental climatic and soil conditions is manifested by a relatively rapid growth of young shoots, but also very often by a rapid discontinuation of growth. The growth of young shoots can continue later, so the one-year growth of young

shoots takes place in 2 or even 3 cycles of growth (Mićić *et al.*, 2018). This leads to the spontaneous development of sylleptic shoots. This phenomenon can also be caused by some measures that are applied during the vegetation (fertilization, irrigation or pruning). Table 1. shows the development of sylleptic shoots in different cultivars of apricots (their number and length), developed after the heading cut of young shoots during the same vegetation.

Table 1. Number and length of sylleptic shoots per one-year-old shoot

Cultivars	Number of sylleptic shoots		Lenght of sylleptic shoots (cm)	
	Heading cut shoots	No heading cut shoots	Heading cut shoots	No heading cut shoots
'Goldrich'	3.44±0.11	0.08±0.00	20.40±1.18	18.95±0.94
'Roxana'	4.05±0.10	0.24±0.00	16.06±0.88	10.25±0.73
'Hungarian Best'	1.90±0.09	0.00	21.04±1.31	/
Average	3.13±0.20 a	0.10±0.01 b	19.17±0.90 a	14.60±0.86 b
ANOVA	**		**	

The number of sylleptic shoots per one-year-old shoot was significantly higher when the young shoots were cut during the vegetation (June, 10) than in case of spontaneous development. The highest number of developed sylleptic shoots, in the variant when the young shoots were pruned, was obtained in the cultivar 'Roxana' (4.05), and the smallest in the cultivar 'Hungarian Best' (1.90). The same regularity was repeated in the variant when the young shoots were not pruned, only the values were significantly lower (0.24 spontaneously developed sylleptic shoots in the cultivar 'Roxana', while in the cultivar 'Hungarian Best' there was no spontaneous development of sylleptic shoots). 'Roxana' is a cultivar that responds well to young shoots cutting during vegetation (Milošević *et al.*, 2011; Glišić and Milošević, 2019), which is confirmed by the results of this work.

The length of the developed sylleptic shoots was significantly higher in the case when the young shoots were pruned (19.17 cm) than when they developed spontaneously (14.60 cm). It depends on numerous factors, such as cultivar, rootstock, irrigation, mineral nutrition. Glisic *et al.* (2015) state that the term of pruning of young shoots during the vegetation significantly influenced the length of developed sylleptic shoots. Also, the length of developed sylleptic shoots can be significantly affected by the place of cutting of young shoots (Glišić *et al.*, 2016).

The characteristics of the flowering phenophase in 2019 of investigated apricot cultivars are shown in Table 2.

Table 2. The flowering phenophase on sylleptic shoots and other types of bearing wood

Cultivar	Flowering phenophase on sylleptic shoots			Flowering phenophase on other types of bearing wood		
	Onset	End	Duration	Onset	End	Duration
'Goldrich'	19 March	25 March	6	16 March	21 March	5
'Roxana'	21 March	26 March	5	19 March	23 March	4
'Hungarian Best'	21 March	26 March	5	18 March	23 March	5

The flowering phenophase is one of the most critical phases in the annual apricot cycle. In 2019, flowering of apricot began on March 16 ('Goldrich'), March 18 ('Hungarian Best') and March 19 ('Roxana') and lasted 4 to 5 days on trees on which the pruning of young shoots was not applied during the previous vegetation. The opening of flower buds on sylleptic shoots developed on trees in which the pruning of the young shoots was applied, was on March 19 ('Goldrich') and March 21, respectively ('Hungarian Best' and 'Roxana'). In 2019,

flowering on sylleptic shoots occurred 2-3 days later than on other types of bearing wood (Figure 1). It is consistent with the results of Milatović (2013), which states that the earliest flowering starts in the spurs ('May bouquets') and short bearing shoots, then the long (mixed) bearing shoots, and at the latest on the sylleptic shoots. This can have great practical significance in the years when late spring frosts occur (Milošević *et al*., 2008; Milatović, 2013).



Figure 1. Difference in flowering time on sylleptic shoots (a) and other types of apricot bearing wood (b)

Well-developed sylleptic shoots, which are differentiated into quality bearing wood by the end of the vegetation, bring quality fruit in the following vegetation. The characteristics of apricot fruits developed on sylleptic shoots and other types of apricot bearing wood are shown in Table 3.

Table 3. Characteristics of apricot fruits developed on sylleptic shoots and other types of bearing wood of apricot varieties

Cultivars	Fruit weight (g)		Stone weight (g)		Flesh percentage (%)	
	Sylleptic shoots	Other types of bearing wood	Sylleptic shoots	Other types of bearing wood	Sylleptic shoots	Other types of bearing wood
'Goldrich'	62.35±3.15	69.26±3.38	4.01±0.14	4.25±0.12	93.56	93.73
'Roxana'	61.80±2.21	74.20±4.50	3.95±0.22	4.12±0.10	93.60	94.44
'Hungarian Best'	55.24±3.39	58.76±2.65	3.60±0.18	3.72±0.09	93.48	93.61
Average	59.79±2.10 b	67.40±3.05 a	3.85±0.18 b	4.04±0.10 a	93.55	93.92
ANOVA	**		*		ns	

Sylleptic shoots of apricot, developed during the second or third wave of growth of young shoots, bear flower buds of medium or even weak degree of differentiation of flower primordia (Mićić and Đurić, 1999). Despite of this, they have a high realization of bearing potential because they bloom later, in more favorable and stable conditions for pollination, fertilization and fruit set (Mićić *et al*., 2018). In our investigation (Table 3), fruits developed on sylleptic shoots had a lower weight (59.79 g) than fruits of the same cultivars developed on other types of bearing wood (67.40 g). The reasons for this may be precisely in the allegations of Mićić *et al*. (2018) that fruiting on sylleptic shoots is at a high level, so in accordance with that, the fruit weight is smaller. 'Goldrich' and 'Roxana' had fruits of higher weight than fruits of the 'Hungarian Best' cultivar, which is in line with the characteristics of the mentioned cultivars (Milatović, 2013). Stone weight is proportional to the fruit weight (larger

fruits had a larger stone and vice versa). Flesh percentage did not differ significantly depending on whether the fruits developed on sylleptic shoots or on other types of bearing wood.

Conclusion

The pruning of young shoots of apricot during the vegetation caused the development of a larger number and longer length of sylleptic shoots than when they formed spontaneously. Apricot flowering on sylleptic shoots was later than on other types of bearing wood. For the 'Goldrich' and 'Hungarian Best', this difference was 3 days, and for the 'Roxana' 2 days. Fruits developed on sylleptic shoots had smaller fruit weight than fruits developed on other types of bearing wood.

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FRUIT QUALITY OF PLUM CULTIVARS DEVELOPED AT THE FRUIT RESEARCH INSTITUTE IN ČAČAK, SERBIA

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Abstract

The experiment evaluated the fruit characteristics (fruit weight, length, width and thickness, and stone weight) and contents of primary metabolites (soluble solids, total sugars, invert sugars, protein, titratable acidity and pH) in the fruit of plum cultivars. Research included fruits of thirteen plum cultivars developed at the Fruit Research Institute in Čačak (Serbia). During the experimental period (2016-2018), significant differences in the parameters tested were observed among the cultivars. The fruit weight ranged from 26.9 g to 67.4 g, and stone weight from 0.89 g to 2.27 g. The fruit and stone weight were the greatest in cultivar 'Timočanka', and the lowest in cultivar 'Zlatka'. The highest value for average fruit length was obtained in 'Timočanka' (52.4 mm) and 'Čačanska Rana' (51.7 mm), fruit width in 'Timočanka'(45.1 mm) and 'Valerija' (46.2 mm), while fruit thickness was the highest in 'Valerija' (44.6 mm). In terms of primary metabolites, cultivars 'Krina' (21.8%), 'Mildora' (21.9%), 'Pozna Plava' (21.2%) and 'Valjevka' (20.9%) had the highest content of soluble solids. The highest total and invert sugar contents were determined in 'Mildora', while 'Mildora', 'Zlatka' and 'Valerija' were found to have highest content of protein. In contrast, the highest values for titratable acidity and pH value were obtained in 'Čačanska Lepotica', and the lowest in 'Mildora' and 'Nada'.

Keywords: *Prunus domestica L., fruit dimensions, primary metabolites.*

Introduction

Plum (*Prunus domestica* L.) is one of the most important and most spread species of cultivated fruit trees in Serbia. The leading place in the structure of cultivars occupied cultivars with combined traits, such as 'Čačanska Lepotica', 'Čačanska Rodna' and 'Stanley'. Čačanska Lepotica' and 'Čačanska Rodna' are Serbian plum cultivars, which along with 15 other ('Boranka', 'Čačanska Rana', 'Valerija', 'Timočanka', 'Čačanska Najbolja', 'Nada', 'Zlatka', 'Mildora', 'Krina', 'Valjevka', 'Čačanski Šećer', 'Pozna Plava', 'Divna', 'Petra' and 'Lana') were developed at the Fruit Research Institute in Čačak, Serbia, during seventy years of work on plum breeding (Lukić *et al.*, 2016). The largest amount of plum fruits produced in Serbia is processed into brandy, while much smaller amounts are dried, frozen, and processed into other products. Fresh consumption of plums is quite small. Due to changes in the plum cultivar assortment, new research concerning the chemical composition of fruits are needed. Namely, plums represent an excellent source of nutrients which contribute significantly to human nutrition and are considered to be one of the most important commodities consumed worldwide. Fruit of plum contains relatively large amounts of carbohydrates, constituting a significant source of available energy (Walkowiak-Tomczak *et al.*, 2008) and have relatively high organic acid content (Gil *et al.*, 2002). Also, in plum juice, sugars reach up to a concentration of 93% of the total soluble solid content (Pereira *et al.*, 2013). Due to the abundance of bioactive compounds such as anthocyanins, pectins and carotenoids plums

constitute a valuable component of our diet, in terms of their nutritive and dietary value (Ionica *et al*., 2012).
Therefore, the objective of this study was to evaluate and compare the fruit characteristics and chemical composition of the tested plum cultivars.

Material and Methods

The orchard trial with plum cultivars was established at the Preljinsko brdo facility of Fruit Research Institute, Čačak (43°55′26″N, 20°26′52″E) near Čačak city (Western Serbia), during 2016-2018. Plum cultivars are grafted on 'Myrobalan' (*Prunus cerasifera* Ehrh.). The analysis involved 13 plum cultivars ('Boranka' 'Čačanska Rana', 'Valerija', 'Čačanska Lepotica', 'Timočanka', 'Čačanska Najbolja', 'Nada', 'Zlatka', 'Mildora', 'Čačanska Rodna', 'Krina', 'Valjevka' and 'Pozna Plava') developed at the Fruit Research Institute, Čačak. Orchard management was consistent with standard cultural practice (summer pruning, fertilization, pests and disease control, irrigation).
The fruits were sampled in the phase of physiological maturity. The trial was conducted in 3 replication with 5 plum trees per replication and 10 plum fruits per replication. Fruit weight and stone weight were determined on a Mettler precision scale with an accuracy of 0.01 g. For each plum fruit, three linear dimensions, length, width and thickness were measured by using a digital caliper with a accuracy of 0.01 cm.
The chemical analysis of the fruit included the following: 1. Soluble solids content was determined by a digital refractometer (Kruss, Germany); 2. Total sugars and invert sugars were analyzed by the Loof-Schoorl method (Egan *et al*., 1981); 3. Titratable acidity was assessed by the 0.1 N NaOH titration method using phenolphthalein as indicator; 4. Protein content was determined by Kjeldahl's method (Helrich, 1990); 5) Actual acidity (pH value) was measured by a pH Meter (Iskra MA 5707, Slovenia).
The experimental data obtained research period were subjected to statistical analysis using Fisher's two-factor analysis of variance - ANOVA. Significant differences between the mean values of the tested factors were determined by LSD test at $P \leq 0.05$ significance levels. The results are presented in figure and tabular form.

Results and Discussion

The results on the fruit and stone weight in plums, as well as demonstration of significant differences among the cultivars, are presented in Figure 1. In our study, fruit weight ranged from 26.9 g to 67.4 g, and stone weight from 0.89 g to 2.27 g. Fruit weight and stone weight were the highest in the cultivar 'Timočanka' (67.4 g and 2.27 g, respectively), and the lowest in the cultivar 'Zlatka' (26.9 g and 0.89 g, respectively). Also, the high fruit and stone weight were detected in 'Čačanska Rana' (50.2 g and 2.26 g, respectively) and 'Valerija' (53.8 g and 2.12 g, respectively). Generally, most of the studied cultivars are characterized by large fruit (more than 30 g). Exceptions are only cultivars: 'Mildora', 'Čačanska Rodna', 'Zlatka' and 'Krina' that had medium large fruit. Significant differences were found between cultivars for fruit dimensions (Table 1). The highest values for fruit length were obtained in 'Timočanka' (52.4 mm) and 'Čačanska Rana' (51.7 mm), fruit width in 'Valerija' (46.2 mm) and 'Timočanka'(45.1 mm), and fruit thickness in 'Valerija' (44.6 mm), whereas the lowest values for fruit length (36.0 mm), width (31.3 mm) and thickness (30.6 mm) were recorded in 'Mildora'.

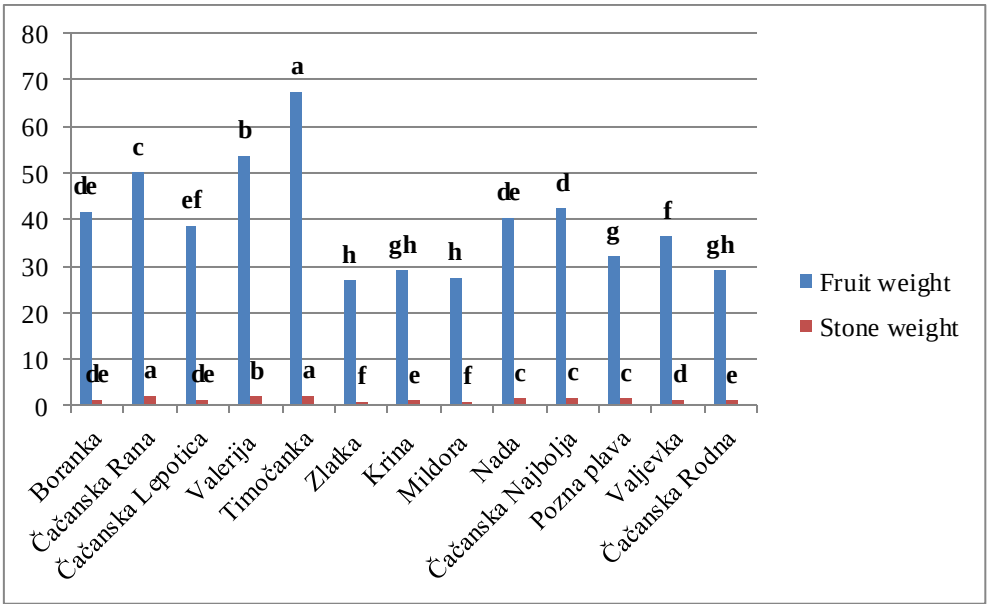


Figure 1. Fruit weight and stone weight of plum cultivars (g)

Compared to the present experiment, under the environmental conditions of Serbia, Mitrović *et al.* (2005), Milatović *et al.* (2011), Miletić *et al.* (2011) and Glišić *et al.* (2016) recorded similar results. On the other hand, the presented data on the mass and dimensions of the fruit have lower values in relation to the data obtained in Montenegro (Božović *et al.*, 2017). These differences can be attributed to the effect of climatic factors on the fruit of the studied plum cultivars.

Table 1. Fruit dimensions of plum cultivars (mm)

Cultivars	Length	Width	Thickness
Boranka	42.4±1.04 e	40.6±0.81 c	39.4±0.79 c
Čačanska Rana	51.7±0.79 a	42.2±0.65 b	39.4±0.86 c
Čačanska Lepotica	43.2±0.56 e	37.9±0.35 d	37.8±0.82 d
Valerija	48.0±0.90 bc	46.2±0.82 a	44.6±0.64 a
Timočanka	52.4±0.87 a	45.1±0.69 a	43.2±0.61 b
Zlatka	44.7±1.06 de	32.9±0.36 f	32.0±0.75 f
Krina	44.2±0.51 de	35.0±0.83 e	32.9±0.45 f
Mildora	36.0±0.33 f	31.3±0.61 g	30.6±0.53 g
Nada	47.8±0.64 bc	38.4±0.28 d	35.6±0.29 e
Čačanska Najbolja	46.5±1.39 cd	40.0±0.95 c	39.2±0.59 cd
Pozna Plava	42.7±0.20 e	33.6±0.26 ef	32.9±0.28 f
Valjevka	46.6±1.69 cd	32.4±0.25 fg	31.6±0.23 fg
Čačanska Rodna	46.1±1.46 cd	37.0±0.85 d	34.8±0.27 e
ANOVA			
Cultivar	**	**	**

Means followed by different letters within the columns are significantly different at P≤0.05 according to LSD test and ANOVA (F-test) results.

Traditional quality properties in fruits are dry matter, sugars, dietary fiber, organic acids and pigments, whereas soluble solids content (consisting mostly of mono- and disaccharides), titratable acidity and juice pH (representative of total acids), contribute to sweetness and acidity of fruits (Skrede *et al.*, 2012). Sugars and acids represent the basic components in the formation of fruit flavor. According to Mikulič-Petkovšek *et al.* (2012), the composition and

contents of sugars and acids affect fruit organoleptic characteristics. The results on the contents of primary metabolites in plum fruits are given in Table 2. It is believed that the high fruit quality and its good flavor are related to the soluble solids content present in the fruit, which in European plums ranges between 12% and 32% (Neumuller, 2010). In the presented results detected amounts of soluble solids varied from 13.4 to 21.9%. The highest content of soluble solids was determined in cultivars 'Krina' (21.8%), 'Mildora' (21.9%), 'Pozna Plava' (21.2%) and 'Valjevka' (20.9%), and the lowest in cultivar 'Valerija' (13.4%). 'Mildora' had the highest content of total sugars (13.45%) and invert sugars (9.64%), as opposed to 'Boranka' (9.10% and 6.52%, respectively), which exhibited the lowest values. Protein content was highest in 'Valerija', 'Zlatka' and 'Mildora' and lowest in 'Pozna Plava'. In contrast, as regards acids, the highest levels for titratable acidity and pH value were obtained in 'Čačanska Lepotica', while the lowest levels of titratable acidity and pH value were recorded in 'Mildora' and 'Nada'. Generally, the highest values for soluble solids, sugars and protein, and the lowest values of titratable acidity and pH value were obtained in cultivar 'Mildora'.

Table 2. Content of primary metabolites in plum fruits

Cultivars	Soluble solids (%)	Total sugar (%)	Invert sugar (%)	Titrability acidity (%)	pH	Proteins (%)
Boranka	14.8±1.03fg	9.10±0.03g	6.52±0.16f	0.96±0.02ab	3.58±0.01e	0.69±0.03d
Čačanska Rana	14.7±0.82fg	9.38±0.11fg	7.20±0.10de	0.97±0.02ab	3.56±0.02e	0.66±0.02d
Čačanska Lepotica	16.0±0.16ef	10.6±0.22de	7.63±0.29cd	0.99±0.01a	3.46±0.01f	0.67±0.03d
Valerija	13.4±0.32g	9.03±0.21g	8.70±0.18b	0.91±0.02bc	3.57±0.03e	0.77±0.02a
Timočanka	17.3±0.50de	9.80±0.24fg	9.08±0.24ab	0.67±0.02e	3.46±0.01f	0.73±0.02b
Zlatka	17.8±0.19cd	10.2±0.13ef	6.78±0.20ef	0.88±0.02c	3.40±0.02fg	0.77±0.02a
Krina	21.8±0.51a	11.5±0.26bc	7.05±0.32de	0.68±0.02e	3.76±0.02ab	0.74±0.02b
Mildora	21.9±0.51a	13.4±0.27a	9.64±0.20a	0.52±0.03f	3.35±0.02g	0.77±0.02a
Nada	19.1±0.42b	11.3±0.35c	6.75±0.25ef	0.57±0.03f	3.69±0.03bc	0.67±0.03d
Čačanska Najbolja	17.5±0.60cd	9.60±0.23fg	8.60±0.26b	0.85±0.02c	3.65±0.03cd	0.66±0.03d
Pozna Plava	21.2±0.63a	11.6±0.39 b	7.54±0.24cd	0.73±0.04d	3.55±0.05ef	0.64±0.03e
Valjevka	20.9±0.23a	11.4±0.32bc	6.87±0.19ef	0.95±0.01ab	3.62±0.05de	0.73±0.03b
Čačanska Rodna	19.3±0.77b	12.5±0.48ab	7.83±0.21c	0.65±0.02e	3.61±0.02de	0.74±0.02b
ANOVA Cultivar	**	**	**	**	**	**

Means followed by different letters within the columns are significantly different at P≤0.05 according to LSD test and ANOVA (F-test) results.

The high content of total sugars in cultivar 'Mildora' identified in our samples, is in accordance with the report Miletić *et al.* (2014) and Milatović *et al.* (2016), who observed that very high contents of soluble solids and low acids in 'Mildora' confirms its suitability for drying. Also, Milatović *et al.* (2016) reported that 'Nada' is characterized by a low acid content and a very sweet taste. Božović *et al.* (2017) recorded higher soluble solids content and sugar but lower acids levels in fruits of plum grown in Montenegro, which was not confirmed in the present study. Generally, the results of the present experiment on the chemical composition of fruits are comparable to the results of Mitrović *et al.* (2006), Miletić *et al.* (2013), Glišić *et al.* (2016) and Tomić *et al.* (2019).

Conclusions

Over the three-year experimental period, examined fruit dimensions were highest in cultivar 'Timočanka', and lowest in cultivars 'Mildora' and 'Zlatka'. The cultivars had high levels of primary metabolites, which directly contributed to fruit quality, therefore can be recommended for fresh consumption, processing and drying. 'Mildora' exhibited excellent chemical characteristics of the fruit, primarily in terms of their soluble solids, sugar and protein, while the other cultivars showed variability in the studied parameters. Given the good fruit dimensions and their chemical composition, the tested cultivars are suitable for growing in Serbia and can be recommended for commercial production in intensive plum orchards.

Acknowledgements

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DYNAMIC OF TOTAL PHENOLIC CONTENT *cv* CABERNET SAUVIGNON DURING RIPENING AND IMPACT ON ANTIOXIDANT CAPACITY OF WINE

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Abstract

In this study, the impact of ripening phenophases on the total phenolic content in wines and their antioxidant capacity was studied. Grapes were harvested in three different stages of maturity: vèraison, optimal enological maturity and overripeness, which originated from vineyards belonging to experimental field "RADMILOVAC" of the Faculty of Agriculture in Zemun, University of Belgrade (Serbia). After grapes crashing and destemming, the samples of crushed grapes sulfited with 10 g of $K_2S_2O_5$ per 100 kg and yeast strain *Saccharomyces cerevisiae* in the amount of 20 g/hl (BDX, Lallemand, Canada) was inoculated. Alcohol fermentation with maceration lasted 21 day at temperature of 25±3□C using the "pigeage"system (mechanically punching down). After that pomace was separated and obtained wine samples were bottled and stored until analyses. Total phenolic content in wine samples was determined by the Folin–Ciocalteu's (FC) method using gallic acid as a standard. According to this method, the highest phenolic content was for vèraison sample (1385.0 mg GAE/l), then for optimal enological maturity sample (1325.0 mg GAE/l), and the lowest content was for overripeness sample (1070.0 mg GAE/l). Also, anti-DPPH radical activity and antioxidant capacity were analysed using FRAP and TEAC methods. The results of these antioxidant methods showed that higher total phenolic content led to better antioxidant capacity of wine samples. A vèraison wine sample showed the highest antioxidant capacity which was positively correlated with their total phenolic content.

Keywords: *Total phenolic content, grape maturation, antioxidant capacity, wine.*

Introduction

Many compounds of enological interest (phenolic compounds, aroma precursors) are known to be accumulated in that tissue throughout ripening (Vicens *et al.*, 2009). As the berries are growing, the sugar content rises, while the concentration of the acids decreases. Furthermore, during vèraison the grapes accumulate color (anthocyanins for red grapes), aroma compounds, tannins and minerals. The moment when the grape is fully ripe, called physiological ripeness, is characterized by maximum sugar content, and it is also the moment when the sugar, acidity and pH levels are in a good balance for harvesting (Ivanova *et al.*, 2011; Milosavljevic, 1998). Accumulation of anthocyanins in the red grape varieties starts at vèraison (Kennedy *et al.*, 2002) while proanthocyanidins are mainly accumulated before vèraison (Cadot *et al.*, 2006). Vèraison is the period when grape berries go through several changes i.e. the green color is changing into yellow-green for the white grapes or into red and different blue nuances for the red grapes due to accumulation of anthocyanins in the skins (Milosavljevic, 1998). The softening mechanism during vèraison may be associated with the changes in polysaccharide composition and structure of the mesocarp cell walls (Yakushiji *et al.*, 2001). Proanthocyanidins are primarily responsible for the astringent properties of red wine (Des Gachons and Kennedy, 2003). It is generally accepted that tannins are not chemically broken

down to any great extent during grape ripening and they just become more bound up within the grape berry and are less easily extracted (Kennedy *et al.*, 2007). At the time of the late harvest the berries are naturally dehydrated, associated with shrinkage of the berries, because they have been left longer on the vine (Ivanova *et al.*, 2011).
The objective of the present paper was to provide information about impact of ripening phenophases (vèraison, optimal enological maturity, overripeness) on the total phenolic content and antioxidant capacity of produced wines.

Materials and methods

The grape variety Cabernet Sauvignon (vintage 2017) was harvested in three different stages of maturity: vèraison, optimal enological matutity and overripeness, which originated from vineyards belonging to experimental field "RADMILOVAC" of the Faculty of Agriculture, University of Belgrade (Serbia). After grapes crashing and destemming, the samples of crushed grapes sulfited with 10 g of $K_2S_2O_5$ per 100 kg and yeast strain *Saccharomyces cerevisiae* in the amount of 20 g/hl (BDX, Lallemand, Canada) was inoculated. The grapes of vèraison strage were harvested at last week of August. Total sugar content was 19.6 % and titratable acidity by titration with NaOH, was 7.5 g/l eqv. tartaric acid. Total sugar content was corrected to 24 % by adding sugar (saccharose). After this stage grapes were harvested at optimal enological maturity at first week of October. Total sugar content was 23.5 % and titratable acidity by titration with NaOH, was 6.8 g/l eqv. tartaric acid. Three weeks after optimal enological maturity, grapes were harvested at overripeness stage, with the highest sugar content (25.1 %) and titratable acidity 4.6 g/l eqv. tartaric acid. Alcohol fermentation with maceration lasted 21 day at temperature of 25±3□C using the "pigeage" system (mechanically punching down). After that pomace was separated and obtained wine samples were bottled and stored until analyses. Total phenolic content in wine samples was determined by the Folin–Ciocalteu's (FC) method using gallic acid as a standard. It was analysed anti-DPPH radical activity and antioxidant capacity using FRAP and TEAC methods.

Results and discussion

There are different external factors that will influence synthesis of total phenolic compounds in the grape berry (temperature, sunlight, soil, irrigation, plant growth regulators, time of vintage during the berry ripening, winemaking process) (Meng *et al.*, 2017; Nel, 2018). Nevertheless, in this study, it was observed that maturity stage of grape has a great influence to total phenolic content of wine (Ivanova *et al.*, 2011; Kennedy *et al.*, 2002). The highest phenolic content was for vèraison sample (1385.0 mg GAE/l), then for optimal enological maturity sample (1325.0 mg GAE/l), and the lowest content was for overripeness sample (1070.0 mg GAE/l) (Figure 1). Several studies have investigated seed polyphenol changes during grape ripening and have found that the amount of extracted polyphenols declines with maturity (Kennedy *et al.*, 2000) and it was in agreement with our investigation. According to Ivanova (2011), before véraison starts accumulation of proanthocyanidins in the red grape varieties and also anthocyanins accumulated at vèraison, so it could describe our findings. Also, it is important length of maceration and extractability of phenols accumulated in that stage of maturity. The softening mechanism during vèraison may be associated with the changes in polysaccharide composition and structure of the mesocarp cell walls (Yakushiji *et al.*, 2001). Insufficiently ripened grapes have a lower extractability of anthocyanins and proanthocyanidins from skins and a higher extractability of proanthocyanidins from seeds, which may produce more astringent and bitter wines (Zhou *et al.*, 2019). The higher the ethanol concentration is, the higher the extraction of

total phenolic compounds from seeds in all the stages of maturity. Its influence is the greatest for skins phenols (Ribereau-Gayon *et al.*, 2006). Tannin synthesis starts very early in berry development and continues until vèraison in skin and for 1–2 weeks after vèraison in seeds. Tannin maturation, resulting in decreased extractability of the tannins, occurs during ripening. During maceration time, it was observed increase of ethanol concentration which facilitate diffusion of total phenolic compounds from solids of grapes. Anthocyanin synthesis occurs in the skin of red grapes after vèraison, and after tannin synthesis of seeds and skin is complete. In grapes, many studies have observed a significant decrease in tannins after vèraison, indicating that the tannins are being modified and are no longer readily extracted which can describe results in our study (Kennedy *et al.*, 2007). The moment when the grape is fully ripe, called physiological ripeness, is characterized by maximum sugar content, and it is also the moment when the sugar, acidity and pH levels are in a good balance for harvesting (Ivanova *et al.*, 2011). The synthesis of anthocyanins starts during vèraison and remains active throughout grape ripening, which causes a gradual accumulation in the skins. In contrast, proanthocyanidin concentration is highest at vèraison and subsequently decreases until just before complete ripeness, after which time it remains relatively constant (Gil *et al*, 2012). Wine produced from grapes harvested at optimal enological maturity was caracterised by a slightly lower total phenolic content because of reaction of copigmentation, oxidative degradation of anthocyanins and polymerisation with moleculs of proanthocyanidins (Blesic, 2016).

In both seeds and skin, there is no tannin synthesis during the later stages of berry ripening when extractable levels of tannin actually decline. They just become more bound up within the grape tissues and are less easily extracted. The decrease in the total phenolic content during the ripening could be a result of the changes of the concentration of esters of hydroxycinnamic acids in skins, as a significant part of the total phenolics. Esters of hydroxycinnamic acids could be oxidized or bonded in complex compounds (for both the white and the red grapes), or they could participate in the synthesis of acetylated anthocyanins (for red grapes) (Kennedy *et al.*, 2000; Ivanova *et al*, 2011).

Antioxidant activity of wine samples produced from grapes harvested in different maturity stages

Wine produced from grapes harvested at vèraison had the highest anti-DPPH radical activity and also antioxidant properties showed by FRAP and TEAC methods. Other two samples, optimal enological maturity and overripeness, had lower values (Table 1). This was confirmed by some authors who investigated correlation of total phenolic content and antioxidant properties (Petrovic *et al.*, 2019; Plavsa *et al.*, 2012) (Figure 2, 3, 4).

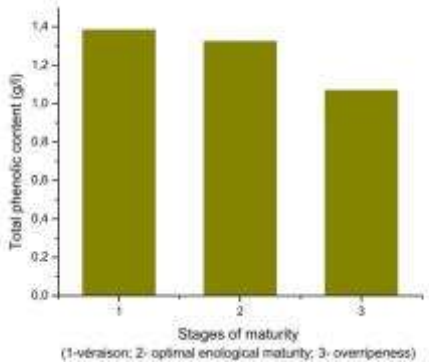


Figure 1. Impact of maturity stages of grapes on total phenolic content of wine.

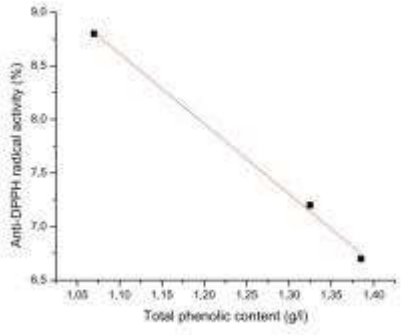


Figure 2. Positive correlation between total phenolic content of wine samples and its anti-DPPH radical activity.

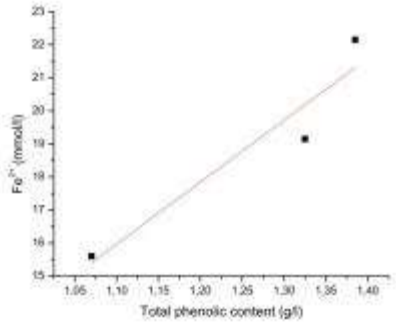


Figure 3. Positive correlation between total phenolic content of wine samples and FRAP values.

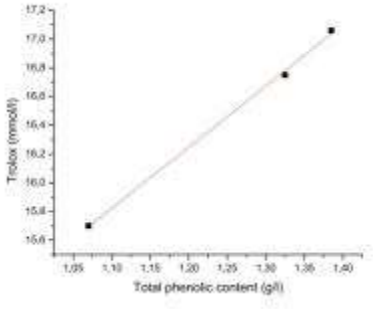


Figure 4. Positive correlation between total phenolic content of wine samples and Trolox values.

Table 1. Values of antioxidant properties of wines produced from grapes at different maturity stages.

Maturity stages	DPPH (%)	FRAP (mmol Fe^{2+}/l)	TEAC (mmol Trolox/l)
Vèraison	6.70	22.15	17.06
Optimal enological maturity	7.20	19.15	16.75
Overripeness	8.80	15.60	15.70

Conclusions

In this paper, the importance of grape ripeness on antioxidant capacity was confirmed. The amount of phenolic compounds in grapes depends on the variety, degree of ripeness, ripening conditions (sunlight, temperature), phytosanitary state and wine making process. The amount that will pass into the wine depends of maceration conditions and the extractibile properties of individual phenols. During the grapes ripening the amount of monomeric phenols will be reduced which affects antioxidant properties of wine.

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INFLUENCE OF IBA BASED STIMULATORS AND IAA BASED BIOSTIMULATOR ON PROPAGATION OF *ROSMARINUS OFFICINALIS* L. BY CUTTINGS

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Abstract

Rosmarinus officinalis L. is a valued perennial medicinal and aromatic plant belonging to *Lamiaceae* family. Vegetative propagation of this plant by cuttings is preferable since its seed is difficult to germinate. The aim of this research was to achieve the highest number of successfully rooted cuttings of *R. officinalis* by the use of various IBA or IAA based stimulators. Prior to putting into trays filled with a mixture of peat moss and perlite, cuttings were treated with the IBA based rooting powders "Chryzotop Green 0.25" (IBA 0,25%), "Rhizopon AA" (IBA 0.5%) and "Rhizopon AA" (IBA 1%), IAA based rooting biostimulator "Slavol S" (0.1-1 mg/L of IAA) and only water (control). The experiment was performed in the laboratory conditions inside a polythene tent for plant propagation under artificial lighting and with the use of the intermittent mist-propagation system. Two evaluations (on the 15th and 30th day) of the rooting of 32 cuttings per treatment were conducted following the placement of cuttings in the trays, and the rooting rate (%) and the absolute root dry mass (mg) were recorded. The first evaluation showed the better efficacy of all IBA stimulators compared to the IAA biostimulator and control, while the second one revealed that treatments with IBA 0.5 and 1% did not differ between themselves and both were the most efficient treatments. Therefore, they could be successfully used as rooting stimulators of *R. officinalis* as the absolute root dry masses were 19.06±12.28 and 14.24±7.46 mg, while the rooting rates were 93.8 and 68.75%, respectively.

Keywords: *rosemary, vegetative propagation, IBA stimulator, IAA biostimulator.*

Introduction

Rosmarinus officinalis L. is a valued perennial medicinal and aromatic plant belonging to the Lamiaceae family. It is highly distributed in the Mediterranean region where it naturally grows as an evergreen, up to 2m high shrubby plant. Rosemary is widely used in making pharmaceutical products for treating a digestive, diuretic, diaphoretic and urinary ailment. Leaves of plant are used are used in culinary, while the leaves and essential oil are used in cosmetic and perfume industry (Stepanović and Radanović, 2011; Haloui *et al.*, 2000; Nicola *et al.*, 2002; Jelačić *et al.*, 2007). It is also grown for ornamental purposes in private gardens, porches, patios and balconies (Iapichino *et al.*, 2006).

In Serbia the predominant cultivation technique for *R. officinalis* is the propagation by cuttings with the application of synthetic stimulators (Jelačić *et al.*, 2007). Apart the fact that vegetative propagation is much faster and simpler technique in comparison to grafting and micropropagation, according to Nicola *et al.* (2002), it is a preferable propagation method in medicinal and aromatic plant species of *Lamiaceae* family such as rosemary since its seed is difficult to germinate and give rise to plants with a minor development.

In a modern plant production, apart from already known synthetic stimulators, microbiological fertilizers and plant development bioregulators are being more and more

utilized (Rafiee *et al.*, 2016; Poincelot, 1993) and some of them have been tested in cultivation of *R. officinalis* (Jelačić *et al.*, 2007; Mahmoud, 1995).
What is new to this study is vegetative propagation of rosemary cuttings performed in laboratory conditions. Usually, vegetative propagation by cuttings is conducted in outdoor conditions or inside greenhouses under the influence of environmental factors. Our experiment was done in conditions similar to one provided in vertical plant production systems where crops can be grown inside growth rooms equipped with electrical lighting. Vertical plant production systems were originally developed in Asia (Japan, Taiwan and Korea) for vegetable production and later have been introduced in the United States (Hartmann and Kester, 2018; Despommier, 2013).
The aim of this research was to achieve the highest number of successfully rooted cuttings of *R. officinalis* by the use of various IBA based stimulators or IAA based biostimulator with popagation being performed in laboratory conditions inside a polythene tent with provided mist system and artificial lighting.

Materials and methods

Origin of plant material: Plant material used for the preparation of stem cuttings were one-year-old shoots taken from the 5-year-old mother stock plants grown inside a plastic greenhouse in the collection of medicinal plants on the production fields of the Institute, in Pančevo (44° 52'20.0" N, 20°42'04.7" E).
Preparation of cuttings: Softwood terminal stem cuttings, 13cm in length, were made. The leaves on the lower portion of the cuttings were removed, leaving 3 to 4 nodes. All cuttings were sterilized in 0.5% sodium hypochlorite for 10 min and then twice quickly rinsed with distilled water. In basal portion, the cuttings were wounded with pruning shears, making 2cm vertical cuts on 2 sides of each cutting.
Treatments: The basal portions of cuttings (3cm) were submitted to 5 different treatments. The cuttings were treated with tree IBA based rooting powders: "Chryzotop Green 0.25%", Rhizopon®, Netherlands (IBA 0.25%), "Rhizopon AA powder 0.5%", Rhizopon®, Netherlands, (IBA 0.5%) and "Rhizopon AA powder 1%", Rhizopon®, Netherlands (IBA 1%). The fourth treatment involved the use of IAA based rooting biostimulator "Slavol S" (contains 0.1- 1 mg/L of IAA) in form of solution prepared according to manufacturer instructions (250 ml "Slavol S" dissolved in 500 ml of tap water), in which cuttings were immersed for 3h. In the fifth, control treatment, the cuttings have been immersed just in tap water.
Research location and growth conditions: The experiment was conducted at the Institute of Medicinal Plant Research "Dr Josif Pančić", in the laboratory of the Department of Agricultural Research and Development located in Belgrade, Serbia (44°49′ N, 20°28′ E) during the period April 18th to May 17th, 2019. Following the application of treatments, the cuttings were transferred into the trays for propagation, filled with a cutting-specific medium (Steckmedium, Klasmann-Deilmann GmbH) of following characteristics provided by the manufacturer: a mix of peat moss and perlite, structure 0 - 6 mm, fertilizer NPK 12:14:24 - 0.5 kg/m^3, pH value 5.5 - 6.5, the EC value 15 mS/m (+/- 25 %).
The trays with cuttings were kept inside a polythene tent (Grow Box), under the following growing conditions: the artificial lighting produced by cool fluorescent tubes (fluorescent Biolux 36W and Flora 36W), with a 16-hour photoperiod, providing up to 4,000 lux ≈ 54 μmol m^{-2} s^{-1} of photosynthetic photon flow density (PPFD); the air humidity of 70 to 90% provided by intermittent automatic mist-propagation system, which was set to operate in following time-dependent modes: from 1st to 10th day - 5s/15 min, from 11th to 20th day – 5s/30 min, and from 21st to 30th day – 5s/90min; the air temperature was from 20°C to 23°C,

while the substrate temperature was kept almost constant (23±2°C) by placing a heating mat below the trays.

The experimental design and followed rooting parameters: The experimental design was a randomized complete design with 32 cuttings per each treatment. Evaluation of the rooting was made on the 15th and 30th day following the transfer of treated cuttings into the trays and it was carried out by determining the rooting rate (%) and by measuring the absolute dry mass of the roots (mg). Each evaluation included all 5 treatments, with 160 cuttings provided in total per evaluation. The rooting rate was calculated by counting the number of the rooted cuttings per treatment and then divided it by the total number of cuttings provided for each treatment and multiply it with 100. The absolute root dry mass was recorded after roots have been dried at 105 °C during 48 hours to a constant mass.

The obtained data were analyzed by analysis of variance (ANOVA) using STATISTICA 7.0. software. Multiple comparisons of treatments were performed by using Tukey test to detect significant differences between the arithmetic means of the absolute dry mass of the roots ($p<0.05$).

Results and Discussion

The evaluation made on the 15th day showed the better efficacy of all IBA stimulators compared to the IAA biostimulator and the control. Also, IAA based biostimulator proved to be more effective compared to the control in which there were no rooted cuttings. Based on the measured masses of the absolute dry roots, all IBA treatments were equally effective. However, when it comes to a number of rooted cuttings, the first evaluation showed that for a 15-day long rooting period under the established conditions, the highest number of successfully rooted cuttings was obtained with the use of IBA 0.25% with 30 rooted cuttings out of 32 cuttings placed on rooting, while IBA 0.5% and IBA 1% treatments had only 16 and 13 successfully propagated cuttings, respectively. The rooting rate for each treatment was presented in detail in Table 1.

Table 1. Rooting rate (%) and absolute root dry mass (mg) of rooted cuttings from *Rosmarinus officinalis*, evaluated on 15th and 30th day following the placement of cuttings in the trays.

Treatment	Evaluation on the 15th day		Evaluation on the 30th day	
	Rooting rate [%]	Root dry mass[a] [mg/ treatment]	Rooting rate [%]	Root dry mass [mg/ treatment]
IBA 0.25 %	93.75	4.98± 3.31^{ab}	93.75	12.52± 8.19^{b}
IBA 0.5 %	50.00	4.51±3.07^{ab}	93.75	19.06±12.28^{a}
IBA 1 %	40.63	6.94±6.17^{a}	68.75	14.24±7.46^{ab}
Slavol S	18.75	1.37±0.83^{b}	87.50	4.78±2.41^{c}
Control	/	/	68.75	4.44±2.00^{c}

[a]Values are presented as means ± standard deviation. Values with the same letter in each column showed no statistically significant difference ($p<0.05$); Statistical analysis was based on a one-way ANOVA and Tukey HSD tests;

The second evaluation, made on the 30th day, revealed that treatments with IBA 0.5 and 1% were both the most efficient with regard to the values of the absolute dry root mass. Therefore, both treatments could be successfully used as rooting stimulators of *R. officinalis*, although it should be emphasized that a higher number of rooted cuttings was achieved with the use of IBA 0.5% compared to IBA 1% stimulator. Similar, Parađiković *el al.* (2013) confirmed that softwood terminal cuttings of rosemary could be successfully rooted with the

use of rooting powder stimulator IBA 0.5% Rhizopon I®. In their study, cuttings of *R. officinalis* were left to root for 14 weeks, which resulted in higher fresh rooted cuttings mass (1.51g) treated with IBA 0.5% compared to those from control treatment (1.24 g), while the rooting rate was 93%.

According to results obtained in this study, the number of rooted cuttings for IBA 0.5% and 1% treatments increased on the 30th day, and they were 30 and 22, with the rooting rates 93.75% and 68.75%, respectively. In study of Ayanoğlu and Özkan (2000), 3 different IBA treatments (in concentrations of 0, 100 and 200 ppm), were applied on *Salvia officinalis* L. cuttings and on 15th and 30th day their rotting rate was also estimated. There were no rooted cuttings on the 15th day in the control treatment, while in IBA 100 ppm treatment the highest rooting rate 35% was observed, and it further increased to 78.75% on 30th day. In our study, the absolute root masses measured on the 30th day, apart from being higher than those gained on 15th day, also indicated differences in efficacy of IBA treatments, where auxin in concentration of 0.5% was more effective than in concentration of 0.25% (Table 1).

Apart from auxin concentration, other factors can also affect rooting of cuttings, such as season during year in which cuttings are put on rooting (Klein *et al.*, 2000; Nicola *et al.*, 2003). In study done by Mehrabani *et al.* (2016), *R. officinalis* cuttings were put on rooting in July, August and September and treated with IBA (0,1000, 2000 and 3000 mgl^{-1}). Rooted cuttings assayed 40th day following the treatment with auxin in concentration of 3000 mgl^{-1}, had the most positive effects on fresh root mass (7.49g) in September, with the highest survival rate of 80%. According to our results, with the similar concentration of IBA 0.25%, on 30th day following the treatment the rooting rate was 93.75 % and dry root mass up to 0.02 g were gained in spring. While, Silva *et al.* (1997), performing the study in Brazil, outstood the end of winter as the best season for rooting *R. officinalis* cuttings and with the use of lower IBA doses (25, 50, 75 and 100 ppm) achieved the dry root masses (0.08, 0.08, 0.02 and 0.03g, respectively) slightly higher than those obtained in our study on 30th day (Table 1).

Although the IAA based biostimulator was not more effective than IBA based stimulators, as in the evaluation made on the 15th day, on the 30th day it gave a higher number of rooted cuttings than the control treatment. Jelačić *et al.* (2007) reported that applied biostimulator "Slavol", in the dose of 5 ml/L of water in intervals of 7 days, made the greatest effect on the root mass (fresh 0.872 g and dry 0.072 g), while in our study on 15th and 30th day achieved values of absolute root dry masses were to 0.002 and 0.007 g, respectively. In study done by Mahmoud (1995), rosemary cuttings were soaked in solutions with lower (of 10 to 100 ppm) and higher doses (of 1000 ppm to 2000 ppm) of natural IAA for 1 and 2 hours which resulted in inhibitory action in the higher doses and optimal root development in cuttings subjected for 1 hour to level of 10 ppm (dry root mass 70.3 mg) or for 2 hour to level of 100 ppm (dry root mass 42.1 mg), indicating that the success in rooting cuttings varies depending on the used concentration and soaking time in IAA application. In our study, IAA based biostimulator, on the 30th day, gave the values of the absolute dry root masses of rooted cuttings that didn't differ from those in control treatment, suggesting the need to investigate other concentrations of IAA and different soaking times of auxin application in future studies.

When it comes to influence of provided propagation environment on rooting of cuttings, in conditions that involved enclosed mist system some studies have been executed. In study done by Shahhoseini *et al.* (2015) in Iran on semi-hardwood cuttings of rosemary, cuttings were treated in solutions of IBA (0, 1000, 2000, 3000, 4000 and 5000 mg/L) for 1 min and held in plastic tunnel under mist system. After 70 days, the highest rooting percentage was obtained at concentration of 5000 mg/L IBA (66%), which is nearly 30% lower than the rooting rate achieved in our study on 30th day with the use of the same concentration of IBA on softwood stem cuttings. Zaharia *et al.* (2015) reported that cuttings of *Labisia pumila* propagated in misting system had better rooting ability (84%) than those in the non-mist

system (72%). In order to test the affect of mist system, studies on propagation of others medicinal plants, in laboratory conditions inside a polythene tent and under the artificial lighting, should be carried out in future.

Conclusion

The results of this study have demonstrated that stem cuttings of rosemary can be successfully propagated in laboratory conditions inside a polythene tent with provided mist system and under the artificial lighting. Application of auxins positively affected rosemary. Calculated rooting rates and measured absolute root day masses on the 30th day proved to be higher than those gained on 15th day, indicated that 30-day long period is the optimal period for rooting of *R. officinalis* in established laboratory conditions with the use of IBA in a concentration of 0.5 and 1%. When it comes to the use of IAA based biostimulators, there is a need for further research regarding the use of other biostimulators in different concentrations and variations in the application methods. Further research shall be extended to more medicinal and aromatic plants with significant economic importance.

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NUTRIENTS AND BIOACTIVE COMPOUNDS OF ALMONDS

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Abstract

The almond *Prunus amygdalus* L., is a species that belongs to the *Amygdalus* subgenus inside the *Prunus* genus, the *Rosaceae* family and the order *Rosales*. It is written in a Bible that sweet almond is one of the oldest cultivated fruit species. It is well adapted and spread throughout the whole Mediterranean region from which 28% of the world production is obtained. Almond is among the most popular tree nuts produced and consumed worldwide as a good dietary source of protein, monosaturated fatty acids, dietary fiber, vitamin E, riboflavin and essential minerals (manganese, magnesium, copper and phosphorus). Daily consumption of 30-50 g of almonds provides macronutrients, micronutrients, and phytochemicals of high biological value. These bioactive compounds have a synergistic effect in preventing and delaying many age-related pathologies (e.g. cardiovascular diseases, stroke, type 2 diabetes mellitus, certain types of cancer, and several neurodegenerative diseases). The consumption of almonds has been scientifically proven to improve life span and health span and should be a part of a healthy diet. The objective of this review was to summarize the latest information of almond nutrients and bioactive compounds so as effect of processing on changes of nuts properties. Health benefits of almond intake were also discussed. Several factors affected the nutritional quality of almonds, including genetic and environmental factors. In addition, the form of consumption additionally changes composition of almonds. The roasting process induced chemical and microstructural changes, especially altering the lipid composition, favoring its oxidation and modifying antioxidant compounds. The major health benefits of almonds were related to its beneficial impact on lipid profile. Around 90% of the lipids were in the unsaturated form which were cardio protective by decreasing low-density lipoprotein (LDL) cholesterol and mildly increasing high density lipoprotein (HDL) cholesterol.

Keywords: *almond, consumption, kernel, health benefits. et al.*

Introduction

The almond *Prunus dulcis* (Miller) D.A. Webb, is a species that belongs to the subgenus *Amygdalus* inside the genus *Prunus*, the *Rosaceae* family and the order *Rosales*. Sweet almond is one of the oldest cultivated fruit species and it is appraised for its culinary uses and numerous medicinal properties. The ancient Greek doctors Hippocrates and Galen described almond as 'hot', and noted its 'heating', 'cleansing', 'nourishing' and 'strengthening mental functions' properties. Almond originates from Central and Southwest Asia. As a food source, which can be easily stored and transported, along with grapes, olives and figs it spreads in the early Bronze Age (around 3000-2000 BC) to the lands of ancient civilizations in the Middle East (Zohary and Hopf, 2000).

Almond is the most important tree nut in the world in terms of commercial production. Based on the FAO data for 2018 (FAOSTAT, 2020), almond production is constantly increasing in the world, and it is currently at about 3.2 million tons (average, in shell). The main area for almond production is the central part of California (the valleys of the Sacramento and San

Jacinto rivers). The United States is the world's largest producer of almonds with over 1.8 million tons or 57.9% of world production and 21% of the total area under almonds The second most important region for almond production includes countries around Mediterranean Sea where 28% of the world production is obtained. Spain is the largest producer, with more than 339,000 tons (10.5% of world production) and about 32% of the world's area under almonds. Significant producers from this region are Italy, Turkey, Morocco, Tunisia, Algeria and Greece. Australia is the largest almond producer the Southern Hemisphere with 2.2% of the global crop. The countries of Central and Southwest Asia (Iran, Syria, Pakistan, Ukraine, Uzbekistan, Afghanistan) also have significant almond production. Almonds are the most consumed tree nut, with a per-capita consumption of 1 lb./y in the USA. Kernels do not contain gluten, so they are often used to prepare meals for people who are allergic to wheat or suffer from celiac disease (gluten intolerance). It is often used as a substitute for breast milk to feed babies who have a lactose problem.

The kernel is the edible part of the almond nut with a high nutritional value (Socias i Company *et al.*, 2008). Besides protein and lipid fraction almonds are source of sugars, mainly fructose and sucrose (Balta *et al.*, 2009), vitamins (Segura *et al.*, 2006) and minerals (Özcan *et al.*, 2011). Various phytochemicals such as phenolic acids, flavonoids, stilbenes and ligants appear in low quantities (Manach *et al.*, 2004) but have large influence on almond quality. As consumers are becoming more interested in healthy life styles, almonds are emerging as some of the most popular edible nuts, typically used as snack foods or as ingredients in a variety of processed foods, especially in bakery and confectionery products (Cordeiro and Monteiro, 2001). Although almonds contain high amounts of fat, the lipid fraction does not contribute to cholesterol formation in humans, due to high level of unsaturated (monounsaturated and polyunsaturated) fatty acids (Askin *et al.*, 2007; Beyhan *et al.*, 2011). Daily consumption of 30-50 g of almonds provides macronutrients, micronutrients, and phytochemicals of high biological value and is advisable to ensure various health benefits without the risk of body weight gain (Vadivel *et al.*, 2012). Numerous clinical and pre-clinical trials and epidemiologic studies showing that regular consumption of almonds can significantly reduce low density lipoprotein (LDL), cholesterol, postprandial glycemia and insulinemia; improve body weight control; and reduce the risk of obesity-related diseases such as coronary heart disease (CHD) and type II diabetes (Hyson *et al.*, 2002; Hollis and Mattes, 2007; Jenkins *et al.*, 2008; Rajaram *et al.*, 2010; Wien *et al.*, 2010; Damasceno *et al.*, 2011). Almond oil extracted from kernels using cold-pressed method contain various vitamins such as vitamin E, B, and A; minerals; and omega fatty acids that make this oil an all-purpose essential and carrier oil which is utilized in food preparation, pharmaceutical and cosmetic industries as well in pharmaceutical and cosmetic industries. The chemical composition of almonds is affected by many factors. It depends both on genotype and also environmental factors such as growing region, cultivation methods, climatic conditions, cultivation methods, and kernel maturity (Sathe *et al.*, 2008; Yada *et al.*, 2011, 2013; Kodad *et al.*, 2014a, b; Muhammad *et al.*, 2015).

Almond nutrients

Lipids and fatty acids

Almonds are a rich source of lipids composed predominantly of monounsaturated (MUFAs) and polyunsaturated (PUFAs) fatty acids (Venkatachalam and Sathe, 2006; Čolić *et al.*, 2017). Lipid content is very important factor in the confectionery industry. Kernels with a high content of lipids can be used to produce nougat or to extract oil, while kernels with a low percentage of lipids are suitable for production of almond milk and almond flour. The lipid fraction is also a major determinant of kernel flavor particularly following roasting (Socias i

Company *et al.*, 2008). Together with moisture level, proteins and oil composition, high lipid content, defines the nutritive value of almonds. As reported by Miraliakbari and Shahidi (2008), oil fraction is composed of various lipid classes, such as triacylglycerols, sterols, sterol esters, phosphotidylserine, phosphotidylinositol, phosphotidylcholine, and sphingolipids. Studies of oil content in commercial or local almond cultivars/selections have been carried out worldwide reporting considerable variability. Oil content is within a range 43–66.1% of kernel fresh weight (FW) and 20-67.5% of kernel dry weight (DW). Conducted studies indicate that oil content depends mainly on the genotype, year as well as, other factors such as growing condition, pollen source (Yada *et al.*, 2013; Kodad *et al.*, 2014b; Alizadeh – Salte *et al.*, 2018), rootstock (Čolić *et al.*, 2018), and harvest time (Matthäus *et al.*, 2018).
Fatty acids in almond are very similar in composition to olive oil and generally considered desirable for a healthy diet. In a recent review, Čolić *et al.* (2019) showed the existence of the same pattern in the majority of almond samples analysed. Oleic, linoleic, palmitic and stearic acids (in decreasing order) represent over 95% of the total fatty acid content.

Protein and amino acids

Among the nut fruits almonds are considered a good source of high-quality proteins. Protein is the second major chemical component of the almond kernels after the lipid fraction. Protein contents of the commercial almond cultivars ranged from 13% to 29% on a dry weight basis (Kodad, 2017). High range of variabiliy depending on the origin of the genotype and the environmental conditioons of the growing region (Kodad, 2013). Almond contains more than 188 proteins (Li and He, 2004), with a good balance of essential amino acids, with exception of methionine, and is easily hydrolyzed by common digestive protease, producing high-quality protein hydrolysates in relation to essential amino acid balance (Sathe, 1993). Recent study of House *et al.* (2019) confirmed lysine as the first□limiting amino acid, while Socias i Company *et al.* (2008) reported glutamic acid, aspartic acid and arginine as the most common amino acids. Protein content is inversely correlated with the lipid fraction and the ratio between these two components is very important for preparation of some processed products. This index shows great variability, ranging from 1.60 to 6.33% (Kodad, 2017).

Carbohydrates and fibre

In almonds, the only carbohydrate that can be digested, absorbed and metabolized by humans to provide a source of energy are sugars, starch and some sugar alcohols. The nonstarch polysaccharides are indigestible and therefore unavailable as an energy source, but they promote physiological effects that are beneficial for human health (Yada *et al.*, 2011). Soluble sugars although present in small amount from 1.8 to 7.6% (Kodad, 2017) are sufficient to make kernels sweet tasting (Schira, 1997). Most soluble sugar is sucrose representing more than 90% ot the total (Socias i Company *et al.*, 2008). Other sugars include rafinose, fructose, sorbitol and inositol (Saura-Calixto *et al.*, 1984; Schira, 1997).
Of all the edible nuts, almonds have the highest total dietary fiber content (Mandalari *et al.*, 2010), consisting of non-starch polysaccharides (cellulose, hemicellulose, pectins, gums, β□ glukans), oligosaccharides, resistant starch and lignin associated with the dietary fiber polysaccharides.

Minerals and vitamins

Almonds are valued as good source of minerals and vitamins. Of the 14 mineral identified as principal minerals for the human body up to nine appear in almond flour in relevant concentrations (Rabadan *et al.*, 2017). Almond kenels are an important source of potassium, phosphorus, calcium, magnesium and manganese (Yada *et al.* 2013; Mahmoud and Yasin, 2016). Potassium content predominates with values ranging from 465 to 1235mg/100 g dry

weight (Drogoudi *et al.*, 2013). The amount of potassium was repoorted to be four times more than amount of sodium (Kodad, 2017). Also, almonds are recognized as a good source of iron, zinc and manganese. Mahmoud and Yasin (2016) observed that for heavy metals the amount of chromium was the highest (4.577 μg ml^{-1}) which is a suitable biochemical nutritional to promote weight loss, muscle development and to treat the symptoms of type 2 diabetes.
Regarding vitamins, almonds are rich in lipid-soluble (E type, with its major homologue α-tocopherol and minor ones γ-tocopherol, β-tocopherol, and α-tocotrienol) and in small amounts of water-soluble (B type, B1, B2, B3, B5, B6, B7, B9) vitamins (Prgomet *et al.*, 2017). Ascorbic acid (vitamin C) and vitamin K were not reported in almond (Yada *et al.*, 2011). Proximate content of ash (which represents the mineral content) in almonds is 3 g ash/100 g FW (Yada *et al.*, 2011).

Bioactive compounds

Epidemiological evidence indicates that the consumption of foodstuffs containing antioxidant phytonutrients such as flavonoids and other polyphenolics is advantageous for our health (Amarowicz *et al.*, 2004). Although almond consumption was traditionally perceived as unhealthy due to its high fat content, recent studies have evidenced the beneficial effects enclosed to the frequent consumption of nuts due to the capacity of their bioactive nutrients and non-nutrients to lower the plasma levels of low-density lipoprotein cholesterol (LDL-C) and the incidence and severity of cardiovascular disorders (Sabate *et al.*, 1999, 2003; Hyson *et al.*, 2002; Berryman *et al.*, 2011). Concerning bioactive non-nutrients, the influence of phenolic compounds and their radical scavenging activity on almond quality and human health have been widely reported (Shahidi, 2004; Blomhoff *et al.*, 2006; Chen *et al.*, 2006). Almond bioactive compounds have a synergistic effect in preventing and delaying many age-related pathologies (e.g. cardiovascular diseases, stroke, type 2 diabetes mellitus, certain types of cancer, and several neurodegenerative diseases).

Phenolic compounds

Among phytochemicals, polyphenols are considered to possess potential bioactivity and health-promoting properties. High variety of phenolic acids and flavonoids have been reported in almonds. Protocatechuic acid was predominant in the majority of published papers (Sang *et al.*, 2002; Milbury *et al.*, 2006; Wijeratne *et al.*, 2006a, b) together with p-hydroxybenzoic acid and vanilic acid. Other phenolic acids included caffeic, p-coumaric, ferulic, sinapic, syringic, gallic, and ellagic acid.
Almond is among the richest sources of nut flavonoids, having 61 mg/100 g (Bolling, 2017). Even through the almond skin represents just around 4.0% of the total almond weight, 60.0–80.0% of the almond phenolic compounds are distributed within the almond skin (Milbury *et al.*, 2006). Eleven out of 19 phenolic compounds identified in almond kernels, skin, and blanch water are found only in the skin, whilst 95.0% of the individual flavonoids identified were also present in the skin (Milbury *et al.*, 2006). Monagas *et al.* (2007) identified a total of 33 compounds corresponding to flavanols, flavonols, dihydroflavonols and flavanones, and other non-flavonoid compounds in almond skin. Within flavanols, the most abundant compounds identified are catechin and epicatechin, while isorhamnetin-3-O-rutinoside and kaempferol-3-O-rutinoside are the major flavonol glycosides present in almond skin (Monagas *et al.*, 2007; Bartolomé *et al.*, 2010). Milbury *et al.* (2006) reported that isorhamnetin (in the form 3-O-glucoside or 3-O-rutinoside), representing around 70.0% of the total, is the predominant flavonoid in most of the varieties assessed so far, with 97.1 ± 2.3% of compounds originating from skin and 2.9 ± 2.3% from kernel.

Sterols

Almond kernels are an important source of phytosterols that are considered essential elements for human health. Dietary phytosterols inhibit cholesterol absorption that potentially lower risk of developing cardiovascular disease (Ntanios *et al.*, 2003). Moreover, phytosterols may protect against the development of colon cancer (Awad and Fink, 2000) and have a positive effect on the human immune system (Carr *et al.*, 2010). Fernández-Cuesta *et al.* (2012) studied almond kernel phytosterol concentrations in 160 cultivars and found range from 1,126 to 2,777 mg kg^{-1}. Study of 23 local almond seedling populations from Morocco (Kodad *et al.*, 2015) showed that phytosterol fraction consisted mainly of β-sitosterol (78.90–87.26%) and Δ5-avenasterol (6.26–13.04%), which together accounted for more than 90% of phytosterols.

Effect of processing on changes of nuts properties

The kernels of sweet almonds are used raw, fried, baked, with or without peel, or used to make almond butter, almond oil and almond milk. Significant differences, as expressed by the ORAC values, were detected between the three types of thermal processing: roasting > blanching + oven-drying > blanching + freeze-drying. The roasting process induced chemical and microstructural changes, especially altering the lipid composition, favoring its oxidation and modifying antioxidant compounds. Garrido *et al.* (2008) reported the antioxidant activity (ORAC values) higher for the roasted samples (0.803 to 1.08 mmol Trolox/g), followed by the samples subjected to blanching + drying (0.398 to 0.575 mmol Trolox/g) and then the blanched (freeze□dried) samples (0.331 to 0.451 mmol Trolox/g).

The highest levels of antioxidants and antioxidant activities were found for almond kernels roasted at 200 °C for 20 min, followed by those roasted at 180 °C for 20 min, significantly higher in comparison with raw almond kernels (Lin *et al.*, 2016). According to Rusu *et al.* (2018) the best processing method for almonds seems to be roasting, especially dry roasting which enhances the antioxidant activity of polyphenols with a positive influence on human well-being.

Health benefits

Nowadays, almonds are recognized for their health-promoting qualities, particularly for their role in reducing risk of cardio-vascular diseases (including stroke and other chronic ailments), which is probably due to the favorable lipid profile and low-glycaemic nature (Lie *et al.* 2011). Also, almonds oil has numerous properties including anti-inflammatory, immunity-boosting, and anti-hepatotoxicity effects (Hyson *et al.*, 2002; Sultana *et al.*, 2007). According to Kurladinsky and Stote (2006), and Chen et al. (2007) daily consumption of almonds reduces the level of bad cholesterol and the occurrence of cardiovascular diseases, as a result of the combined action of polyphenols from the skin and vitamin E from the oil. After this discovery, almonds were included in all cholesterol-lowering diets. Almond kernels should be consumed daily in the recommended amount of 56 g (Yanagisawa *et al.*, 2006) with pelicle, because it has a polyphenol content of 650-850 ng, which is about 10 times more than in the kernel itself (Wijeratne *et al.*, 2006 a). Almonds are an excellent source of folic acid, that lowers the level of homocysteine, which causes the deposition of fat on the walls of arteries. Significant amounts of copper and iron in almond help the development of blood cells, while magnesium helps prevent heart attacks. Sang *et al.* (2002) were the first to find that almond kernels contain sphingolipids that reduce the risk of colon cancer. Almonds are natural sources of tocopherol, folate, mono and polyunsaturated fatty acids, and polyphenols. These nutrients may prevent or delay the onset of age-related cognitive disorders and amnesia. Rat studies have shown that almonds, when given for 28 days, significantly improved memory retention (Batool *et al.*, 2016). Experiments on human bone cell lines (osteoclasts) have revealed that almond meal can inhibit osteoclast formation. It also interferes with the gene expression and functioning of osteoclasts (Platt *et al.*, 2011). The study by Mandalari *et al.*

(2008) indicates that almond kernels have potential prebiotic properties, i.e. that consumption creates favorable conditions for increasing the levels of beneficial bacteria in the intestines. This is a very important fact, bearing in mind that 80% of immunity depends on diet. Daily consumption of almonds raises the body's immunity. In order to gain the most health benefits whole roasted almonds should be incorporated into diet.

Conclusions

Almond is considered as an important food crop, with a high nutritional value and health-promoting qualities, particularly for his role in reducing risk of cardio-vascular diseases. The consumption of almonds has been scientifically proven to improve life span and health span and should be a part of a healthy diet. Increase in worldwide demand for almond products, high kernel price, moderate production costs, classify almonds in the group of fruit trees profitable for cultivation. Due to global warming, with a gradual rise in mean temperature, almond production is relocating to higher latitudes. Research conducted in Serbia since 2000 demonstrated that it is possible to grow almond in some localities. Therefore it is necessary to foster introduction of new, late flowering almond cultivars and research activities to establish almond orchards and expand of almond culture to the Balkan countries.

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THE EFFECT OF FERTILISATION ON THE PHENOLOGICAL CHARACTERISTICS OF SELECTED APPLE VARIETIES IN SARAJEVO CONDITIONS (BOSNIA AND HERZEGOVINA)

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Abstract

This research has studied the effect of fertilization on the variability of blossom time of different apple varieties in the geographical region of Sarajevo (Bosnia and Herzegovina) in 2020. For sustainable fertility it is necessary to recognize the phenophases of development of different apple varieties and, on that basis, to ensure norms of fertiliser, when and in which manner has to be applied. The research focused on three apple varieties: 'Jonagold_Dacoste', 'Red Idared' and 'Gala Shniga'. Fertilization models applied included the following fertilization treatments: control (no fertilization), standard conventional fertilization (300kg/ha NPK 6-18-36 + 150kg/ha KAN), foliar fertilization (Firofert Crystal 10-40-10+1MgO+ME and Fitofert Crystal 20-20-20+ME with an addition of sucrose solution in the values of 10, 15 and 20%). The goal of the research was to determine the effect of different models and versions of fertilization on the blossom phenophase of the selected apple varieties in the area of Sarajevo, in order to supplement the deficiencies in fertilization arising in the time of fruiting. The results of the research show that different applications of fertilization have a varying effect on the variability of time and scope of blossoming of the selected apple varieties. The most significant average volume of blossoming has been registered for the apple variety 'Red Idared' (grade scale 0-5, grade 5), and the least significant for the apple variety Jonagold_Dacoste (grade scale 0-5, grade 2).

Key words: *apple varieties, phenological observations, fertilisation, pollen germination.*

Introduction

When it comes to volumes of production, apple is the most significant continental fruit type in the world. In the last several years, the apple production has experienced a slight increase and in 2018 it amounted to 83,139,326 tons (FAO 2018). China takes the lead in the produced apple volumes (33.3 million tons), which makes up nearly a half of the world's production, followed by the USA (4.3 million tons). Apple production in EU is at approximately 12.6 million tones, which makes up 20% of the worldwide production (Eurostat, 2006). The largest European apple producers are Poland (2.6 million tons) and Italy (2.2 million tons). In 2019, in Bosnia and Herzegovina, 64.000 tones of apple was produced(Agency for statistics of Bosnia and Herzegovina 2019). Such a significant position of the apple in the world of fruit production is the consequence of the possibility of growing it in different soils and different conditions, especially its nutritional values. Apple can vegitate in temperatures ranging between 45 to -35oC, but it can also be successfully grown in temperature ranges from 35 to -25 oC (Hanić, et al.. 2009). In terms of climate conditions, temperature has the largest effect (absolute minimum, absolute maximum and average daily). Low temperatures have the most significant effect on the pheno-phase development. Some varieties respond differently to the changes in temperatures, and are differently resistant to cold. In addition, low temperature sensitivity is different depending on the season (Đorđević,

2018). Technologically speaking, apple is a demanding fruit sort, being extremely accumulative. The apple sorti s dictated by the market and follows agro-technological growth measures, in particular, the highly-intensive planting in dense circuit with anti-hail protection and irrigation systems followed by quality standard introduction.
Yield quality, their colour, taste, size and firmnes are the variety characteristics of an apple. The mentioned characteristics are affected by ecological factors, especially agro-technical measures, with particular mention of fertilisation effect and its application at different growth pheno-phases and apple development. Depending on the applicable does and ratio of certain types of fertiliser, balanced fertilisation can improve or in the case of their incompetent application to worsen quality of yield (Lukić, 2010).
When it comes to mandatory elements yield quality is to the largest extent affected by: nitrogen, potassium, phosphorus, calcium, magnesium and boron. The lack or excess thereof may negatively affect the quality of the yield. Based on phenological observations, it is possible to approximate the time of awakening of the apple trees, predict blossom and budding times (Waisi et al., 2014). When first buds and leaves appear the apple trees' root absorption of food is insufficient to satisfy the blossoming and other apple pheno-phases and at the very end the total yield (Vučetić, 2012). The balance between growth and apple yield is possible to achieve by applying different agro-technological manuevers, primarily by fertilising at early development phases of an apple. For this reason, it is necessary to experimentaly determine and intervene with adequate fertilisation with the aim of sable, sustainable and quality yield.

Material and Methods

The research was conducted in the locality of the experimental field of the Federal Institute for Agriculture in Sarajevo (Bosnia and Herzegovina), at the collective apple orchards. The age of the apple orchard is 16 years. The training system of the apple is slender spindle. The basic characteristics of the soil in which the experiment was set up were: pH in water 7.0, pH in KCL 6.14; content of N 0.16%; content of CaCO3 0.4%; content of hummus 2.26%; P2O5 28 mg/100g; K2O 21.22 mg/100g. Soil analysis was done in the Institute for agropedology of the FBiH in accordance with the standards BAS ISO 10390: 2005, pH, $CaCO_3$, content of N based on BAS ISO 11261: 2000, content of P_2O_5 i K_2O Al-method. The effect of fertiliser model on the percentage of budding and blossoms of three apple varieties was carried out:′Jonagold Dacoste′, ′Red Idared′, ′Gala Shniga′ grafted to a weakly lush vegetative rootstock M9. The distance between rows is 3.6m, and between plants 1.3m.
The following fertilization methods were used:

- standard conventional fertilisation 300kg/ha NPK 6-18-36 + 150kg/ha KAN (completed in early spring 03. March. 2020)
- foliar fertilisation (Firofert Crystal 10-40-10+1MgO+ME – repeated twice (28. March. 2020. and 14. April. 2020) and Fitofert Crystal 20-20-20+ME -one application in blossom phase (21. April. 2020.)
- control without fertilization.

The experiment was set up using a randomized block system of random distribution in three repeat turns with fertilization variations capturing 9 apple trees. Significance was tested on the basis of LSD test for significance levels 1% and 5%. The results were analyzed using bi-factor analysis (ANOVA) via SPSS 4.5 software.

Results and Discussion

Blossoming dynamic (start of blossoming, full blossom, end and volume of blossom) of the examined apple varieties depended on the variety type, applied fertiliser and weather conditions. The hereditary characteristics of the variety and weather conditions affect the beginning of blossoming of the apple. Changes in air temperature, which to the large extent controls the phenological blossoming dynamic changed the usual blossom dates of the examined apple varieties. The drop in air temperature, which to the largest extent controls the phenological dynamic of all fruit varieties, including apples, moves the blossom date. According to Mišić (1994), the air temperature reduces with elevation for 0.5 – 0.60C/100m. The analysis of the distribution of the most significant phenological phases represents a mandatory element of any re-ionisation (Đorđević,. 2018).

Table 1. Fertilisation models and effect on flowering pheno-phase of different apple varieties

Cultivar	Fertilisation method	Starr of blossom	Full blossom	End of blossom	Length (days)	Blossom volumes (0-5)
		Datum				
Jonagold Dacoste	KAN+NPK	19. April	25. April	05. May	16	3
	Foliar	19. April	24. April	05. May	16	2
	Control	19. April	25. April	05. May	16	2
Red Idared	KAN+NPK	15. April	23. April	02. May	17	5
	Foliar	15. April	21. April	02. May	17	5
	Control	15. April	21. April	02. May	17	5
Gala Shniga	KAN+NPK	21. April	28. April	05. May	14	3
	Foliar	21. April	28. April	05. May	14	3
	Control	21. April	28. April	05. May	14	3

Knowing the blossom times of apple sorts is necessary in order to find adequate combinations of varieties, which produce the optimal possibility for dusting and fertilisation. Blossoming is an extremely critical pheno-phase, so knowing the blossoming times can lower the damaging effect of late spring frost to exposed open blossoms. As a rule, the blossoming phase is shorter at high air temperatures, low percipitation, relatively low humidity and windy weather (Lukić, 2012).

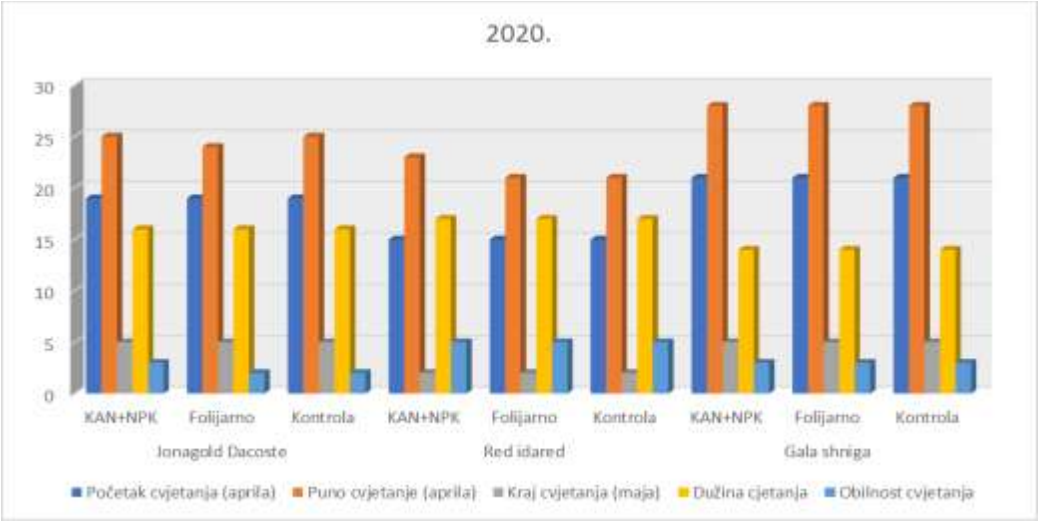


Figure 1. Timeline of different varieties' blossoms

Analysis of the varieties for the beginning of blossoms it can be concluded that different types of fertilisation showed a statistical significance, while the varieties and the interaction between the varieties and the fertiliser did not result in a statistical significance. Generally, the first beginning of blossoming was recorded for variety 'Red Idared' (15. April), then for 'Jonagold Dacoste' (19. April) and the last for 'Gala Shniga' (21. April).

Table 2. Variation analysis for beginning of blossoms

ANOVA *Source of Variation*	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	1,14E-13	2	-5,68	5,68E-14	1	3,554557
Columns	168	2	84	84	7,4E-10	3,554557
Interaction	-1,1E-13	4	-2,8	-2,8E-14	#NUM!	2,927744
Within	18	18	1			
Total	186	26				

On the basis of variance analysis for full blossoms, it can be concluded that the different varieties affected the full blossom while the fertilizer, fertilisation and the variety interaction did not result in a statistically significant outcome. Full blossom was first recorded for variety 'Red Idared' (21. April) with the treatment of foliar fertilisation and the control, while the fertiliseation treatment KAN+NPK identified full blossom (23. April). Apple sort 'Gala Shniga' noted full blossom in both fertilisation treatments and last for the control (28. April).

Table 3. Full blossom variation analysis

ANOVA *Source of Variation*	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	180,6667	2	90,33333	90,33333	4,11E-10	3,554557
Columns	4,666667	2	2,333333	2,333333	0,125592	3,554557
Interaction	5,333333	4	1,333333	1,333333	0,295786	2,927744
Within	18	18	1			
Total	208,6667	26				

Variance analysis for end of blossom showed that the different variants of fertilization affected the end of blossom phase, while the variety, and the interaction between the variant of fertilization did not show a statistically significant outcome. Based on a bi-factor analysis of the variances, it can be concluded that the feriliser type showed to have a statistically significant effect on the length of blossom of the tested apple varieties. Variety as a factor showed statistical significance, while the interaction the varieties and the substrate did not have a statistical significance.

Table 4. Variance analysis for blossom end

ANOVA *Source of Variation*	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	54	2	27	27	3,81E	3,554557
Columns	0	2	0	0	1	3,554557
Interaction	0	4	0	0	1	2,927744
Within	18	18	1			
Total	72	26				

Table 5. Variance analysis for length of blossom phase

ANOVA *Source of Variation*	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	78	2	39	39	2,86E-07	3,5545
Columns	8	2	4	0	0,036534	3,5545
Interact.	4	4	1	0	0,433165	2,9277
Within	18	18	1			
Total	108	26				

Analysing the blossom volumes, it can be concluded that variety as a factor in the variance analysis showed to have a statistical effect, while fertilisation type, and the variety interaction and fertilizer type did not show statistical significance.

Conclusion

In the context of the defined research goal, the following conclusions can be made:

- Factors of apple variety and fertilisation method have a stastistically significant effect on the blossoming phases of apples.
- Blossom dynamic (start of blossoming, full blossom, end and volume of blossom) of apples: 'Jonagold Dacoste', 'Red Idared', 'Gala Shniga', depended on the variety type, applied fertilizer and weather conditions.
- Earliest blossoming was recorded for Red Idared by applying KAN+NPK with foliar fertilization, and the latest for Gala Shniga.
- Full blossom was recorded the earliest for 'Red Idared' with application of KAN+NPK with foliar fertilization, and the latest for 'Gala Shniga'.
- End of blossom was recorded for 'Red Idared', then for 'Jonagold Dacoste' and 'Gala Shniga' with applied fertilizer KAN+NPK.
- Different types of fertilisation affected the end of blossom, while variety, interaction of varieties and fertilizer did not show statistical significance.
- Phenophases of apple blossoms have a timeline depending on the variety, applied fertiliser and are manifested differently in terms of timing.

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INFLUENCE OF GROWTH RETARDANT REGALIS ON BLACKBERRY FRUIT QUALITY

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Abstract

In the group of berry species, blackberry is, by economic importance for Republic of Serbia, immediately behind raspberry and strawberry. As a consequence of the hyper- production on one side and the uncertain sales prospects on the other side, the average blackberry production in Serbia has declined in recent years to approximately 12,000 t (spread to app. 15,000 individual farms). The blackberry orchards in Serbia are dominated by the Čačanska Bestrna and Thornfree cultivars, with more than 95% share, followed by the Black Satin, Dirksen Thornless and some more recent cultivars, such as Loch Ness, Chester Thornless, Triple Crown. The intensification of the cultivation technology accompanied by the introduction of the application growth retardant resulted in increasing the share of quality fruits, i.e. reducing vigour of bush. The paper presents results of a two-year study (2018–2019) into the impact of foliar application Regalis (growth retardant) on morphometric and chemical properties of blackberry fruit. It was observed that most of the tested parameters were affected by foliar application of retardant during the research, as well as by their interaction. In terms of the morphometric properties, weight (10.89 g), height (34.14 mm), and width (26.90 mm) were higher in treatment with Regalis than treatment without application. All chemical properties tested, soluble solids (9.95%), total sugars (4.98%), inverted sugars (4.71%) content, except sucrose and total acids content had higher values in the same treatment. In addition to this, the foliar application of Regalis had a positive effect on the content of secondary metabolities (total anthocianins-67.11 mg/100 g FW; total phenols-636.06 mg/100 g FW), and antioxidant capacity of fruit (2.53mmol Trolox/100 g FW).

Key words: *physical traits, primary metabolities, phenols, blackberries.*

Introduction

The economic significance of the European blackberry (*Rubus* subg. *Rubus* Watson) makes it an important berry fruit in Serbia, where it is positioned immediately after raspberry and strawberry (Nikolić and Milivojević, 2015). The blackberry production constitutes 69% of the European and 17.82% of the world's production (Strik et al., 2014), which places Serbia among the four leading global producers of this fruit. In recent years (2012–2018), according to the data from the Republic Statistic Department (www.stat.gov.rs), blackberry production in the Republic of Serbia significantly oscillated. Semi-erect thornless blackberries (*Rubus* subgenus *Rubus* Watson), such as the two cultivars investigated in this study, have a clearly defined growth cycle (Milivojević et al., 2017). These biennial-fruiting cultivars possess an intensive vegetative primocane growth in the first year. Primocanes grow in length and buds develop in the leaf axils. In temperate regions, cane extension growth diminishes in late summer or fall and process of 'flower initiation' begins in axillary buds on the primocanes (Takeda et al., 2003). The following year, when reproductive growth resumes, these canes produce flowers and become floricanes. Fruiting branches emerge from axillary buds on main and lateral branches of floricanes, which die out soon after fruiting and should be removed at

any time before spring. The intensification of the cultivation technology accompanied by the introduction of the application growth retardant resulted in increasing the share of quality fruits, i.e. reducing vigour of bush. Plant growth retardants are applied to agronomic and horticultural crops to reduce unwanted longitudinal shoot growth without lowering plant productivity. Regalis (prohexadione-Ca) is a new-generation gibberellin biosynthesis inhibitor that has low toxicity and persistence in the plant (Mandemaker et al., 2005). Research of Milivojević et al. (2017) indicated that ProCa on 'Loch Ness' and 'Triple Crown' blackberries reduced the cane and internode length, but increased the node number per meter of cane length. Acording to Poledica et al. (2012), ProCa treatment also significantly reduced cane length and diameter of raspberry 'Willamette', whereas node number per meter of cane length and yield per cane were significantly increased. Factors defining outer and inner fruit quality, such as fruit weight, morphometric traits, and nutritive quality, are also indirectly affected by vegetative growth. The objective of this investigation was to study indirect impact of foliar application with Regalis on fruit quality of Čačanska Bestrna blackberry cultivars.

Material and Methods

Experimental design

Fully ripe fruits of cultivated blackberry of the *Rubus* subg. *Rubus* Watson, species represented by Čačanska Bestrna variety were analised for the morphometric and chemical properties. They have been cultivated in experimental orchards near Čačak (43° 53'N, 20°20'E, 290 m alt.), Western Serbia. The orchards were established in the autumn 2007 and menaged according to standard cultivation protocols (pruning, fertilization, chemical protection, weed control) for intensive production, except irrigation. This region characterised by the mean growing season temperature of 18.2° C and total rainfall of 423.4 mm. The blackberry canes were treated in 2018 with foliar sprays of Regalis containing as an active ingredient ProCa (BASF 125 10 W–10% ProCa, BASF, Germany). Foliar application of Regalis was carried out twice. The first application was performed on 15 April when the primocane growth reached 15 cm in height. A second application of Regalis was done 2 weeks later. The following concentrations of Regalis were applied 1 g 10 L^{-1} in first application and 2 g 10 L^{-1} in second application. The trial was conducted using a randomised block design and it included four replications of each treatment. The treatments consisted of bushes treated with Regalis and untreated (control) bushes. Four selected bushes per replication were investigated (16 bushes per treatment).

Plant material

Blackberry 'Čačanska Bestrna' was created in 1984 by crossing 'Dirksen Thornless' × 'Black Satin' at the Fruit Research Institute, Čačak. It is a highly vigorous cultivar, producing 4–5 strong canes bent in mid-section, having short internodes. The flowering season is mid-late. It is a self-fertile, abundant cropper. Resistant to low winter temperatures, it is medium resistant to yellow rust (*Kuehneola uredinis* (Link)) and susceptible to purple blotch of blackberry (*Septocyta ruborum* (Lib.) Petrark). Ripening season is mid-early, at the beginning of the third decade of July. The fruit is large, with an average fruit weight of around 9.3 g. It is elongated-cylindrical, glossy black, with a sweet-subacidic taste and pronounced aroma. 'Čačanska Bestrna' is suitable for both fresh use and freezing and for various forms of processing as well. In different agro-ecological conditions, this cultivar has displayed excellent performance in respect of cropping and resistance to diseases and low winter temperatures.

Determination of fruit quality traits

Twenty-five fruits in each replication were randomly selected to determine average fruit weight using the Metler balance (±0.01g accuracy) and the data were expressed in g per fruit. The fruit dimensions (mm) (length, and breadth) were also determined in the selected samples using the 'Inox' vernier scales (±0.05 mm accuracy). Chemical parameters viz. soluble solids

content (SSC), total sugars (TS), reducing sugars (RS), titratable acidity (TA), pH, and sucrose (SC) were measured at the commercial maturity stage. SS (oBrix) was determined using a hand refractometer (ATC, Belgium). TS (%) and RS (%) were determined as fresh weight basis, using the Luff-Schoorl method. TA was measured by neutralization to pH 7.0 with 0.1N NaOH, the data being presented as percentage of malic acid. Juice pH was measured using a pH meter (Cyber scan 510, Mettler Toledo, USA). SC was calculated by multiplying the difference of the total and reducing sugars contents by the 0.95 coefficient. The total phenolic content was determined using a modified Folin-Ciocalteu colorimetric method (Singleton et al., 1999; Liu et al., 2002). The monomeric anthocyanin pigment content of the aqueous extracts was determined using the previously described pH-differential method (Torre and Barritt, 1977; Liu et al., 2002). Antioxidant capacity was determined by the ABTS assays according to Arnao et al. (1999).

Statistical analysis

The results are presented as mean ± standard error of mean (SE). Differences between mean values were compared by LSD test in two-way analysis of variance (ANOVA) using MSTAT-C statistical computer package (Michigan State University, East Lansing, MI, USA). Differences with p values of ≤ 0.05 were considered insignificant.

Results and discussion

Within the morphometric characteristic of the fruits of the studied blackberry cultivar, the results of examining fruit weight and dimensions (length and width) are shown (Table 1), in the function of the foliar application of Regalis over the two-year period of trial. The variance analysis showed that the foliar application and the year made a statistically significant impact on the height blackberry fruit, whereas their interaction made a significant impact on the weight, height, and the width of fruit. Fruit weight, height, and breadth ranged from 9.80±0.05 to 12.27±0.11g, 25.33±0.28 to 34.14mm, and 21.69±0.25 to 26.90±0.18mm, respectively.

Table 1. Effect of foliar application of Regalis on morphometric properties of blackberry fruits 'Čačanska Bestrna'

Treatmant		Weight (*g*)	Dimensions of fruit	
			Height (*mm*)	Width (*mm*)
Foliar application (A)				
Regalis		10.89±0.25 a	34.14±12.22 a	26.90±0.72 a
Without application		9.98±0.40 b	30.69±0.80 b	23.55±0.37 b
Year (B)				
2018		11.07±0.58 a	34.21±1.59 a	23.16±0.52 a
2019		11.03±0.59 a	28.02±1.52 b	22.69±0.76 a
A × B				
Regalis	2018	11.97±0.32 a	25.74±22.64 b	24.19±0.20 a
	2019	12.27±0.43 a	25.33±1.84 b	21.69±0.99 b
Without application	2018	10.17±0.87 b	30.98±1.49 a	22.13±0.49 b
	2019	9.80±0.12 b	30.70±0.99 a	21.70±0.62 b
ANOVA				
A		*	*	*
B		ns	*	ns
A × B		*	*	*

Values within each column followed by the same small letter are insignificantly different at the $p \leq 0.05$ by LSD test; ns - non significant differences.

The comparison of with the application and without application showed that the morphometric traits of fruit (weight, height, and breadth) were higher in the application with Regalis treatment. The weight, height, and width were the higher in first growing year. The obtained results of the morphometric properties of the blackberry cultivar examined in this paper indicate that the foliar application of Regalis made a positive impact on the fruit weight and dimensions, with significant differences occurring in the all traits. The obtained results were in agreement with the results of Milivojević et al. (2017) who also, by foliar treatment with ProCa in the plantings of blackberry 'Čačanska Bestrna' and 'Triple Crown', obtained fruits of higher weight, but also a larger number of stones of fruits.

Table 2. Effect of foliar application of Regalis on chemical properties of blackberry fruits 'Čačanska Bestrna'

Treatmant		SSC (%)	Sugars (%)			TA (% of malic acid)
			Total	Inverted	Sucrose	
Foliar application (A)						
Regalis		10.55±0.53 a	4.93±0.11 a	4.67±0.11 a	0.24±0.02 b	1.25±0.01 b
Without application		9.70±0.56 a	4.57±0.01 b	4.11±0.04 b	0.41±0,03 a	1.59±0.04 a
Year (B)						
2018		11.47±0.72 a	4.87±0.14 a	4.56±0.16 a	0.30±0,02 b	1.45±0.10 a
2019		10.78±0.32 a	4.61±0.03 b	4.22±0.09 b	0.35±0,06 a	1.39±0.05 b
A × B						
Regalis	2018	11.77±1.13 a	5.18±0.01 a	4.91±0.01 a	0.25±0,01 c	1.22±0.01 c
	2019	11.33±0.20 a	4.67±0.09 b	4.42±1.14 b	0.22±0,02 c	1.27±0.09 c
Without application	2018	11.17±1.12 a	4.56±0.03 b	4.20±0.09 c	0.34±0,04 b	1.67±1.10 a
	2019	10.23±0.33 a	4.55±0.01 b	4.02±0.07 d	0,48±0,08 a	1.50±0.07 b
ANOVA						
A		ns	*	*	*	*
B		ns	*	*	*	*
A × B		ns	*	*	*	*

Values within each column followed by the same small letter are insignificantly different at the $p \leq 0.05$ by LSD test; ns - non significant differences.

Chemical parameters analysed so far showed a significant effect of foliar application Regalis. On other hand, growing years significantly affected the content of total (TS) and inverted sugars (IS), sucrose (SC), and total acids (TA), except of soluble solids content (SSC) (Table 2). Interaction effect of foliar application and the growing year significantly affected all the chemical parameters, except SSC. SSC, TS, IS, SC, TA in fruit ranged from 10.23±0.04 to 11.77±0.07^{O} Brix, 4.55±0.06 to 5.18±0.21%, 4.02±0.03 to 4.91±0.19%, 0.22±0.04 to 0.48±0.06%, and 1.22±0.03 to 1.67±0.07% of malic acid, respectively. Higher chemical parameters, except SC and TA were recorded in the foliar application with Regalis. The average values of the soluble solids content in all the treatments were higher than the values obtained Miletić et al. (2006) for the same cultivar, while at the same time lower in comparison with the values obtained by Stanisavljević (1999). Observed by years of examination, higher values of chemical properties of the fruit were determined in the first year (2018), except for SC. The analysis of interaction effect of foliar application and growing year (Table 2) inferred that SSC, TS, and IS were the highest in the foliar application with Regalis during the first year, while TA were the highest in treatment without Regalis in the first year and SC were the highest in the same treatment in the second year. Based on the obtained results, it can be concluded that the structure of total sugars is dominated by invert sugars (glucose and fructose), with the fluctuation of the values reflecting the same tendency as the

total sugars. The average values of the total sugars obtained in all of the treatments were higher than the values obtained by Miletić et al. (2006) and Veberic et al. (2014).
Analysis of variance showed significant effect of foliar application on total phenolic content (TPC) where the growing year significantly affected the total anthocyanins content (TAN). The interaction effect of foliar application and growing year showed non significant differences among the examined chemical parameters.

Table 3. Effect of foliar application of Regalis on total antioxidant capacity and, content of secondary metabolities of blackberry fruits 'Čačanska Bestrna'

Treatment		TPH	TAN	TAC
		mg GA/100 g FW		Trolox, mmol/100 g FW
Foliar application (A)	Regalis	805.17±32.12 a	72.42±2.55 a	2.55±0.05 a
	control	631.57±12.33 b	69.80±3.23 a	2.56±0.06 a
Year (B)	2018	466.94±23.69 a	75.85±1.56 a	2.68±0.16 a
	2019	427.94.24±2.29 a	64.17±0.92 b	2.70±0.05 a
ANOVA				
A		*	ns	ns
B		ns	*	ns
A × B		ns	ns	ns

Values within each column followed by the same small letter are insignificantly different at the $p \leq 0.05$ by Duncan's Multiple Range test
ns - non significant differences
control – without application Regalis
FW – fresh weight.

The TAC (total antioxidant capacity), TPH and TAN in blackberries ranged from 2.55±0.05 to 2.70±0.05 Trolox *mmol/100 g* FW, 427.94±2.29 to 805.17±32.12 and 64.17±0.92 to 75.85±1.56 *mg/100 g* FW , respectively. The higher chemical parameters, TAN and, TPH were recorded in the first growing year and TAC in the second year. Comparing the foliar application and without application, it can be concluded that higher TPH and TAN were recorded with application, whereas the control treatment produced higher TAC.
In this study the TAC of blackberries was generally lower than the reported by Moyer et al. (2002), Sellaappan et al. (2002), Siriwoharn et al. (2004) and Clark et al. (2002). The comparison of the differences in TPH and TAN related to the growing year revealed that these were higher in the first than in the second year, suggesting that the growing season, climate and region have an influence on the antioxidant power of blackberries (Sellapan et al., 2002). In the study conducted by Wang and Lin (2000) the total TPH content of berries and leaves varied from 91 to 338 *mg/100 g* of FW. Our results revealed a higher TPH content in 'Čačanska Bestrna' blackberry than that reported by Milivojević et al. (2011) for the same cultivar under similar agro-ecological conditions. On the other hand, Benvenuti et al. (2004) reported that content of TPH in some thornless blackberry cultivars grown in Italy ranged from 192.8 to 351.7 *mg/100 g* FW, which is similar to the results of our study.

Conclusion

In the current study conducted on blackberries, Regalis was primarily used as a plant growth regulator which expressed a positive effect on fruiting of floricanes, and even on morphometric and chemical fruit properties.

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SOME BIO-MORPHOLOGICAL CHARACTERISTICS OF BURLEY TOBACCO VARIETIES AND LINES IN THE PRODUCTION REGION OF PRILEP (NORTH MACEDONIA)

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Abstract

Throughout 2018 and 2019 trials were conducted on the fields of the Scientific Tobacco Institute – Prilep, with four burley varieties (Enchu Ø, B-D-1, Banket A_1, Habana – 92 and two domestic male sterile hybrid lines, B-210/15 CMS F_1 and B-199/11 CMS F_1. The American fertile variety Enchu Ø was used as a control. The examinations were conducted on diluvial soil, using randomized block at 90 x 50 cm spacing design with 4 replications and the obtained results were statistically processed by variance analysis method and were tested with LSD test. During the growing season, morphological measurements were made on 20 stalks of each variety, and the following data were analyzed measurements were performed after harvesting; after each leaf from different stalk position reaches technological maturity: length and width of the 5^{th}, 10^{th} and 15^{th} leaf. In most of these characteristics, the male home varieties proved to be superior to the fertile variety Enchu Ø, and the male sterile hybrid lines proved to be superior to the fertile genotypes B-199/11 CMS F_1 and B-210/15 CMS F_1 created in the Scientific Tobacco Institute - Prilep, North Macedonia. Its high yields and typical Burley characteristics make this male sterile hybrid lines attractive both for farmers and for manufacturers. In the present conditions, it can play the role of an initial variety for restarting the production of Burley tobacco in North Macedonia, but it can also be interesting for our neighboring countries and beyond. The obtained results can help producers make a decision in regard to the genotype that they will use in the start of the production cycle.

Keywords: *tobacco, variety, Burley tobacco, leaves.*

Introduction

The morphological characteristics of each type or variety are more or less different and are genetically controlled. The experiences in this field so far show that in tobacco plants of a specific type, under the influence of inappropriate agro-ecological conditions and agrotechnics, strong variations may also emerge both in terms of the bio-morphological properties, but also in terms of the chemical composition of the obtained raw material. Such variations in certain properties occur despite genetic control, and this phenomenon is known as "plasticity" of tobacco. Therefore, before starting the production of a specific tobacco type or variety, it is necessary to be familiar with the basic prerequisites for its stable production, without major variations in regard to the morphological characteristics and, consequently, the production characteristics of the tobacco. Burley tobacco participates with approximately 30-40% in the composition of blended cigarettes. For that particular reason, in addition to the cultivation of oriental tobacco varieties, large-leaf tobacco types have been grown in North Macedonia as well. The first steps for the introduction of the Burley type in the type structure of the Republic of North Macedonia were made by Rudolf Gornik. The same author (1985) notes that this type of tobacco can only be successfully grown on rich soils and humid climates with frequent rainfall. At the beginning of the seventies extensive efforts were made

to find a variety of this type of tobacco whose major characteristics (primarily yield and quality) would prove to be the best. Uzunoski (1985) indicates that the Burley type dries in shady indoor space, it is an integral part of the mixture for blended cigarettes, it is used for pipes and as a chewing tobacco. A characteristic of the Burley is the light, spongy tissue with a great ability to absorb liquid substances. The content of nicotine and protein is high, and as a result of the long drying process, it has low sugar content, in traces. Its taste is sharp (expressed ammonia-like taste), it is used for the production of American blended cigarettes, and participates in the mixture with as much as 30% (Stankovic, 2002, Georgiev, 2002, Radojčić, 2011). Several Burley varieties of tobacco were created in the Scientific Tobacco Institute - Prilep, which with their yield and quality guarantee profitable production. Since the creation of new tobacco varieties from this and other types of tobacco in the Scientific Tobacco Institute - Prilep is a long-term program commitment, the continuity of the creation of new varieties of tobacco has not been interrupted to date. Hence, a part of these examinations (the morphological properties of the varieties) through the obtained results will be presented in the following sections of this paper.

Material and Methods

The trails included six varieties (lines) of domestic and foreign assortment. The American fertile variety Enchu Ø (USA) was used as the control variety, and in addition to this variety, the tests also included the varieties B-D-1 (Serbia), Banket A_1 (USA), Habana-92 (USA) as well as two domestic hybrid male sterile lines (B-210/15 CMS F_1, B-199/11 CMS F_1), developed at the Scientific Tobacco Institute - Prilep. The trial was set within the Scientific Tobacco Institute – Prilep, on colluvial soil. In the two years of examination the first plowing was performed in the autumn at a depth of approximately 40 cm. In spring, the land was fertilized with artificial fertilizer NPK 8:22:20 with 300 kg/ha and it were additionally plowed twice. The healthy seedlings were transplanted manually in 4 repetitions and with a random arrangement of the varieties in the repetitions with a planting density of 90 × 50 cm (Size of plots is 8.10 m^2, and number of plants per plot is 33). Due to the poor nitrogen supply in the soil before the second hoeing, supplemental nutrition was provided with 5 g/stalk of 26% KAN. The number and quantities of additional irrigation (3-5 with 30 - 40 l/m^2 each) were determined depending on the climatic conditions. The morphological measurements were performed on 20 plants per rep that were typical representatives of the varieties, of the leaves from the lower and the middle belt (5^{th}, 10^{th} and 15^{th} leaf) whereby the length and the width of the leaves were measured, from which the average value was later calculated. Morphological measurements were made during the vegetation phase at the technological maturity of the leaves. The measurements were made on 20 plants of each variety and lines.
During the vegetation, the tobacco was regularly treated with approved chemicals and preporations. The obtained results were statistically processed with the variance analysis method and comparisons were performed with an LSD test (Najceska, 2002, Filiposki 2011).

Results and Discussion

- Length of the 5^{th}, 10^{th} and 15^{th} leaf

In terms of its position, the 5^{th} leaf belongs to the lower harvesting belt, however according to the dimensions of the leaf, it can significantly affect the yield of the tobacco. From the presented data in Table 1 is observable that the variety B - 199/11 CMS F_1 has the largest average length of 61.4 cm. This is 22.2 cm longer than the standard variety where the length is 39.2 cm. In the other trial lines, the length of this leaf is within the range from 53.2 cm in

the B-D-1 variety, 47.6 in the Banket A_1 vaiety to 47.5 cm in HABANA-92 and 56.9 in B-210/15 CMS F_1.

According to the position, the tenth leaf belongs to the middle harvesting belt, and the leaves are usually the largest in the zone where it is formed. From the presented data in this Table 1, it can be seen that the line B - 199/11 CMS F_1 has the largest average length of the 10^{th} leaf of 73.4 cm. That is 23.0 cm longer than the standard variety, which has an average length of 50.4 cm of the tenth leaf. In the other varieties and lines in the trial, the length of this analyzed leaf is within the range from 67.4 cm in the B-210/15 CMS F_1 line to 58.8 cm in the Habana 92 variety.

Risteski et al., (2012) reported that the variety Pelagonec CMS F_1 has the largest average length of the 10^{th} leaf of 66.2 cm.

The fifteenth leaf is also located in the middle harvesting belt of the stalk and it is a highly valued raw material in fabrication. The data in this Table 1 shows that the largest average length of the 15^{th} leaf, of 65.3 cm is a characteristic of the line B-199/11 CMS F_1. This value is 13.1 cm more compared to the control variety where the average length is 52.2 cm. In the Habana-92 variety, the length of this leaf is 53.4 cm, which is 1.5 cm more compared to the control variety. In the other varieties and lines in the experiment, the average length of the 15^{th} leaf is in the range of 65.2 cm at B-204/10, up to 53.5 cm at the Kentucky 14.

Risteski et al. (2009) found that the variety Pelagonec CMS F_1 has the largest average length of the 15^{th} leaf of 62.6 cm.

Table 1. Length of the 5^{th}, 10^{th} and 15^{th} leaf of burley tobacco

Variety	Year	Length of the 5^{th} leaf			Length of the 10^{th} leaf			Length of the 15^{th} leaf		
		Length cm	Average 2018/19	Difference in cm	Length cm	Average 2018/19	Difference in cm	Length cm	Average 2018/19	Difference in cm
Enchu Ø (USA)	2018	39.2	39.2	/	49.3	50.4	/	52.2	52.2	/
	2019	39.2			51.5			52.2		
B-D-1 (Serbia)	2018	55.0^{++}	53.2	+14.0	61.8^{++}	62.3	+11.9	58.4^{+}	59.7	+7.5
	2019	51.4^{++}			62.8^{++}			61.0^{++}		
Banket A_1 (USA)	2018	47.9^{++}	47.6	+8.4	59.8^{++}	60.5	+10.1	55.6	56.1	+3.9
	2019	47.3^{++}			61.2^{++}			56.6^{+}		
HABANA -92	2018	47.4^{++}	47.5	+8.3	58.4^{++}	58.8	+8.4	52.8	53.4	+1.2
	2019	47.6^{++}			59.2^{++}			54.0		
B-210/15 CMS F_1	2018	56.6^{++}	56.9	+17.7	66.6^{++}	67.4	+17.0	60.8^{++}	61.6	+9.4
	2019	57.2^{++}			68.2^{++}			62.4^{++}		
B-199/11 CMS F_1	2018	61.7^{++}	61.4	+22.2	73.1^{++}	73.4	+23.0	64.8^{++}	65.3	+13.1
	2019	61.1^{++}			73.7^{++}			65.8^{++}		

	Length of the 5^{th} leaf		Length of the 10^{th} leaf		Length of the 15^{th} leaf	
	2018	2019	2018	2019	2018	2019
LSD 5% $^{+}$ =	5.14 cm	4.24 cm	4.35 cm	1.81 cm	5.22 cm	3.99 cm
1% $^{++}$ =	7.11 cm	5.87 cm	6.03 cm	2.51 cm	7.23 cm	5.52 cm

The leaves of the 15th leaf in some varieties of tobacco in the study showed not so large statistical differences.

- Width of the 5^{th}, 10^{th} and 15^{th} leaf

The characteristics of the 5^{th}, the 10^{th} and the 15^{th} leaf in the varieties are displayed in Table 2. From the above data regarding the width of the 5^{th} leaf, it can be seen that the variety B –

199/11 CMS F_1 has the largest average width of 40.9 cm. This is 17.0 cm longer than the control variety where this indicator is 23.9 cm. In the other varieties in the trial, the width of the 5th leaf is within the range from 39.3 cm in the variety B-210/15 CMS F_1 to 26.0 in the variety Habana-92. The tenth leaf locally belongs to the middle harvesting belt, and the leaves are usually the largest in the zone where it is formed. The largest average width of the 10th leaf, of 41.4 cm, is a characteristic of the newly created lines B-199/11 CMS F_1 and B-210/15 CMS F_1, which is 14.4 cm more than the control variety where the width of this leaf is 27.1 cm. In the other varieties in this experiment, the width of the 10th leaf is within the range from 39.9 cm in the B-D-1 variety to 32.3 cm in the Habana-92 variety. The highest average width of the 15th leaf of 36.6 cm was registered in the variety B-210/15 CMS F_1, which is 10.2 cm wider than the standard variety, where this value is 26.4 cm. In the other varieties in the experiment, the width of the 15th leaf is within the range from 35.7 cm in the B-199/11 CMS F_1 variety, to 31.6 cm in the Banket A_1 variety.

Table 2. Width of 5th, 10th and 15th leaf of burley tobacco

Variety	Year	Width of 5th leaf			Width of 10th leaf			Width of 15th leaf		
		Width cm	Average 2018/19	Difference in cm	Width cm	Average 2018/19	Difference in cm	Width cm	Average 2018/19	Difference in cm
Enchu Ø (USA)	2018	24.0	23.9	/	26.6	27.1	/	26.6	26.4	/
	2019	23.8			27.6			26.2		
B-D-1 (Serbia)	2018	35.8^{++}	34.3	+10.4	38.6^{++}	39.9	+12.8	34.8^{++}	34.8	+8.4
	2019	32.8^{++}			41.2^{++}			34.8		
Banket A_1 (USA)	2018	30.4^{++}	30.3	+6.4	36.0^{++}	37.1	+10.0	30.6^{+}	31.6	+5.2
	2019	30.3^{++}			38.2^{++}			32.7		
HABAN A-92	2018	25.8	26.0	+2.1	32.6^{++}	32.3	+5.2	27.2	27.0	+0.6
	2019	26.2			32.0^{++}			26.9		
B-210/15 CMS F_1	2018	38.1^{++}	39.3	+15.4	41.6^{++}	41.5	+14.4	36.0^{++}	36.6	+10.2
	2019	40.5^{++}			41.4^{+}			37.2^{++}		
B-199/11 CMS F_1	2018	41.1^{++}	40.9	+17.0	40.6^{++}	41.5	+14.4	35.6^{++}	35.7	+9.3
	2019	40.7^{++}			42.4^{++}			35.8^{++}		

	Width of 5th leaf		Width of 10th leaf		Width of 15th leaf	
	2018	2019	2018	2019	2018	2019
LSD 5% $^{+}$	4.25 cm	1.97 cm	2.28 cm	4.10 cm	3.87 cm	2.36 cm
1% $^{++}$	5.88 cm	2.73 cm	3.17cm	5.67 cm	5.08 cm	3.27 cm

Conclusions

The results of the two-year research (2018/19) generally lead to the following conclusions:

- The longest length of the 5th, 10th and 15th leaf is a characteristic of the line B-199/11 CMS F_1, whereby the length of the 5th leaf is 61.4 cm, and the 10th leaf is 73.4 cm long and the 15th leaf is 65.3 cm highlight.
- The width of the 5th leaf has the highest value in the line B-199/11 CMS F_1 and amounts to 40.9 cm, while the 10th leaf has the greatest width of 41.5 cm in both newly obtained lines (B-199/11 CMS F_1 and B-210/15 CMS F_1). The width of the 15th leaf was greatest in the line B-199/11 CMS F_1.

- The obtained results from the tests show that the line B-199/11 CMS F_1 is dominant compared to the other varieties in the trial.
- The results of the examined properties suggest the conclusion that the newly examined lines are typical of the Burley variety and good results can be achieved in the agro-environmental conditions in the region of Prilep only by applying appropriate agro-technical measures.

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EFFECT OF DIFFERENT CUTTING INTERVALS ON THE FORAGE YIELD AND SOME SILAGE QUALITY CHARACTERISTICS OF GIANT KING GRASS (*Pennisetum hybridum*) UNDER MEDITERRANEAN CLIMATIC CONDITIONS

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Abstract

This study was conducted in Bornova experimental fields of Department of Field Crops, Faculty of Agriculture, University of Ege, Turkey during 2015 and 2016, in order to determine the effect of different cutting intervals (30-, 60-, 90-, 120-, 150- and 180- days) on the forage yield and some quality properties of giant king grass (*Pennisetum hybridum*). Paraíso cultivar of giant king grass (GKG) was used as crop material. Some characteristics were measured such as dry matter (DM) yield, concentrations of crude protein (CP), NDF and ADF. Results indicated that there were significant effects of cutting intervals on the yields and some silage quality characteristics of GKG, and, CP content decreased as inter-cutting interval increased. It was recommended that the production of GKG using 60- day intervals were the most successful cutting frequency regarding the forage yield and quality to the regions with Mediterranean-type climates under irrigation.

Keywords: *Pennisetum hybridum, cutting interval, DM yield, forage quality*

Introduction

Giant king grass (*Pennisetum hybridum*) cultivation under field conditions in Mediterranean environment may be one of those alternatives to produce large amount of high quality roughage (feed) instead of corn. GKG as an interspecific hybrid (6n) has been formed between Napier grass (*Pennisetum purpureum*) (2n=4x=28 chromosomes) and pearl millet (*Pennisetum glaucum*) (2n=2x=14 chromosomes) the resulting hybrid is sterile due to the triploid condition (2n=3x=21 chromosomes) and restored by chromosome duplication with the use of colchicine (Geren and Kavut, 2015). GKG also known as 'Maralfalfa' is a perennial forage grass with a high growth rate, high productivity and good nutritive value and it is mostly used for 'cut and carry' system over the tropical and sub-tropical areas of the world (Wadi et al., 2004). GKG is very palatable to animals at the early leafy stage, but not much liked after stem development.

Cutting interval or frequency between cuts after crop establishment is the common agronomic factor, which affect growth characteristics, DM yield and nutritional quality of perennial forage grasses like *Pennisetum hybridum*, *P. purpureum*, *Miscanthus sp.* etc. (Lounglawan et al., 2014). The harvesting frequency may vary by climatic conditions but should coincide with the timing of high forage quality and the duration between two cuttings depending on the time needed by the plant to regrow (Wadi et al., 2004). Generally, it has been found that the extending cutting intervals increase the DM yield, the higher to proportion of stem and the higher the crude fiber concentration. Digestibility, CP concentration and proportion of green leaf blade drop correspondingly (Campos et al., 2002).

The regrowth after cutting practices is another important physiological process and it determines the herbage quality. Wijitphan et al. (2009) reported that DM yield of Napier grass was increased by extending the intervals of cutting from 4- to 7- weeks. In another study, Mohammad et al. (1988) obtained highest DM yield of 3.3 t ha^{-1} from Napier grass clipped at 8- week interval.

However, before recommending giant king grass as forage crop to farmers, it is essential to understand and determine the relationships between growth characteristics, DM yield, nutritional quality and management practices such as frequency of defoliation to maintain growth of GKG. The objective of this research was to evaluate the influence of different cutting intervals on the DM yield, some yield and silage quality components of GKG under irrigated conditions of Mediterranean climate.

Material and Methods

The experiment was carried out during two growing seasons (2015 and 2016) at Bornova experimental fields of Agricultural Faculty of Ege University, Izmir, Turkey, at about 20 m above sea level with typical Mediterranean climate characteristics (Table 1). The soil was a silty-clay loam (30.6% clay, 36.7% silt, and 32.7% sand) with pH 7.32, organic matter 1.16%, salt 0.074%, 0.123% total N, available phosphorus (1.4 ppm) and available potassium (350 ppm).

Table 1. Some meteorological parameters of experimental area at Bornova in 2015 and 2016

	------- Average temperature (°C) -------			------- Total precipitation (mm) -------		
	2015	2016	LYA	2015	2016	LYA
January	8.9	8.8	9.0	125.1	232.9	112.2
February	9.5	13.8	9.2	101.9	84.9	99.7
March	11.7	13.7	11.8	75.6	122.0	82.9
April	15.9	18.9	16.1	46.4	28.4	46.4
May	20.8	20.7	21.0	30.9	37.1	25.4
June	25.6	27.5	26.0	9.8	2.8	7.5
July	28.0	29.3	28.3	1.8	-	2.1
August	27.7	28.9	27.9	2.6	0.4	1.7
September	23.7	24.7	23.9	15.0	8.6	19.9
October	18.8	19.4	19.1	45.3	0.5	43.2
November	14.0	14.2	13.8	94.8	123.8	109.7
December	10.6	7.1	10.5	141.1	20.2	137.9
✕ - Σ	17.9	18.9	18.1	690.3	661.6	688.6

LYA: Long years average, ✕: Mean, Σ: Total

Six years old "Paraíso" cultivar of GKG was used as crop material. The experiment was carried out with a randomized complete block design with three replications. Each plot was consisted of four rows with 70 cm among the rows and five-meter length (14 m^2). Six different cutting frequencies (30-, 60-, 90-, 120-, 150- and 180- days intervals) were tested.

The recommended dose of 210 kg N ha^{-1} was applied for all plots in three equal doses in every growing season (Mohammad et al., 1988). All plots were fertilised using 80 kg ha^{-1} P_2O_5 and 80 kg ha^{-1} K_2O. ⅓ of N and full dose of P and K fertilization was applied by helping of drip irrigation system at the beginning of April. The other ⅓ doses of N were applied in 30th June and 30th August, in both experimental years, respectively. Drip irrigation system was installed on the field during growing seasons. No weed control was performed during growing years; except the plots cut every 30- day (hand hoeing was done). No evident crop diseases or insects were detected. Harvest was started at 30th of May, and continued at the end of October in both experimental years.

Forage on a central area of three m^2 in each plot was cut at 15 cm above ground level and fresh weight recorded. Harvested fresh forage (2 kg) was dried to a constant weight at 105ºC during 24 h for calculating DM. In each plot, 2 kg chopped samples were vacuum-packed into polythene bags (Geren and Kavut, 2015) with addition of 0.5% salt. No inoculant was applied to any combination. The vacuum bag silos were kept in storage without light for 45 days for anaerobic fermentation. Matured silage samples of each component were dried at 65ºC for 48 h. The dried samples were reassembled and ground in a mill passed through a 1 mm screen. CP contents of

silages were determined using the Kjeldahl method (N %) with a conversion factor of 6.25. The neutral detergent fibre (NDF) and acid detergent fibre (ADF) concentrations were analysed by the sequential detergent analysis method (Goering and VanSoest, 1970).
All data were statistically analyzed using analysis of variance (ANOVA) with the Statistical Analysis System (SAS, 1998). In each cutting frequency, the sum of DM yield and the average of the other characteristics were evaluated. Probabilities equal to or less than 0.05 were considered significant. If ANOVA indicated differences between treatment means, a LSD test was performed to separate them (Stell et al., 1997).

Results and Discussion

The experimental area (Bornova) is characterized by a typical Mediterranean climate with hot and dry summers and cool and humid winters that generally prevailed during both experimental years (2015 and 2016) and long term average. However, as illustrated in Table 1, monthly total precipitation was substantially variable between 2015 and 2016. In both years, air temperature was similar to each other and long year's average. Field studies were started on 6 years old GKG in April by increasing temperature and then satisfactory moisture levels supported by drip irrigation, therefore, stands were excellent. No winter injury on the crops was detected during winter period of the experimental years. We obtained "6, 3, 2, 1+(60- day growth), 1+(30- day growth), 1" number of cuts for intervals 30-, 60-, 90-, 120-, 150- and 180- days in each year in our study, respectively (Table 2).

Table 2. Effects of cutting intervals on DM yield and some forage quality components of GKG.

	2015	2016	2015	2016	2015	2016	2015	2016
Cutting interval	DM yield ($t \cdot ha^{-1}$)		CP content (%)		NDF (%)		ADF (%)	
30- day	19.75	16.28	11.8	12.0	54.6	53.6	37.5	36.7
60- day	42.55	42.03	8.1	8.6	63.6	64.0	40.8	40.5
90- day	39.31	40.76	6.1	6.2	64.6	68.4	44.6	41.7
120- day	38.92	40.34	8.0	8.0	65.1	64.7	42.5	40.9
150- day	44.25	43.40	8.6	9.0	63.1	60.5	40.8	40.6
180- day	51.18	51.51	4.5	5.0	73.9	72.1	46.7	46.4
Mean	39.33	39.05	7.8	8.1	64.1	63.9	42.2	41.1
LSD (.05)	Y:ns CI:1.91 YxCI :ns		Y:ns CI:0.3 YxCI:0.5		Y:ns CI:0.7 YxCI:0.9		Y:0.4 CI:0.7 YxCI:1.0	

Y: year, CI: cutting interval, YxCI: interaction, ns: not significant.

DM yield was not affected by interaction. There were statistically significant differences among cutting intervals regarding DM yield per hectare (Table 2). The highest average DM yield of GKG was obtained from 180- day interval (2015: 51.18 and 2016: 51.51 $t \cdot ha^{-1}$), whereas the lowest average yield obtained from 30- days interval (2015: 19.75 and 2016: 16.28 $t \cdot ha^{-1}$). However, there was no significant difference between 150- and 60- day intervals in both years. Year effect was not significant on DM yield. In the present study, as inter-cutting interval increased from 30- to 60- days, total DM yield of GKG increased, and then decreased to the 120-day interval, while peak DM yields were reached at the 180- day inter-cutting interval. Although, dead leaf material in the sward increased after 120-day interval in the first cut of each cutting regime in both year, while it was limited 90- day interval, there was no dead material in 30- or 60-day interval. Loss of dead leaf is not determined in our study, but variable quantities of dead leaf were observed on the crop, and this has possibly contributed to increased variation in measured DM yield. This suggests that for practical purposes harvest ages above 60 days may be too long

under the conditions of the experiments. On the other side, the rates of regrowth on the total DM yield for 120- and 150- day cutting intervals were approximately 7% and 3%, respectively.

DM yield variation in perennial warm season grasses has been attributed to many factors such as climatic conditions, soil water availability, nutrients availability, plant density, harvest time and method, etc. (Campos et al., 2002; Wadi et al., 2004). Some cutting experiments with GKG also revealed in tropic countries that the choice of cutting interval or frequency is crucial to their performance and were found to be the main factor affecting growth, yield and persistence of swards. In addition to that, many researcher emphasized that DM yield in tropical grasses was positively correlated with plant height, mean tiller weight, crop growth rate and cutting management (Zewdu, 2008; Wijitphan et al., 2009). Tegami Neto and Mello (2007) reported that there was a significant difference in DM yield between 30- and 60- day cutting intervals in *P. hybridum* in Brazil, and DM yield was higher in the plants at a 60- day (78.26 t ha^{-1}) than at a 30- day interval (43.36 t ha^{-1}). Magalhães et al. (2004) informed that DM yield of *P. purpureum* in Brazil significantly increased in line with plant age at cutting, with age at cutting of 84- days yielding the best results (18.93 t ha^{-1}), followed by 56- (9.48 t ha^{-1}), and 42- days (5.02 t ha^{-1}). Another experiment in Japan by Wadi et al. (2004) pointed out that annual DM yield of *Pennisetum* species was higher in the plants at a 90- day (22.6 t ha^{-1}) than at a 60- day interval (14.8 t ha^{-1}). Our findings are in accordance with those researcher's results.

CP content of silage of GKG was affected by interaction (Table 2). The highest average CP content (12.0%) recorded at 30- day interval in 2016, whereas the lowest CP content was 4.5% at 180- day interval in 2015. Mean CP content was significantly higher in second year (8.1%) than the first years (7.8%).

Findings indicated that cutting practices (interval) affected CP content of GKG significantly. However, CP content declined from 11.8-12.0% to 4.5%-5.0 as inter-cutting interval increased from 30- to 180- days in both years, respectively. It is widely reported that CP content decreases as cutting interval increases (Carvalho et al., 2000; Tessema et al., 2010). For example, Carvalho et al. (2000) informed that the CP content of *P. purpureum* decreased as the plant maturity progressed (28-, 35-, 42-, 49-, 56-, 63- days), however they were always above 7% in Brazil. This is related to a decrease in N concentration as plant maturation. In the present study, average CP contents of 120- or 150- days were higher than 60- or 90- days. The reason of mini increase in 120- or 150- days were their second regrowth which had relatively high CP content. The current results are in agreement with Rengsirikul et al. (2011) who found that cutting *P. purpureum* grass 1- month interval obtained the average of CP 12.5%, when the cutting interval extended to 2-, 3- or 6- months, the average of CP decreased to 11.3, 9.4 and 8.1%, respectively. In addition, Tegami Neto and Mello (2007) who found that range of the CP content of GKG were 13.0-11.5% and 10.2-11.9, when cut 30- or 60- days of cutting interval in Brazil, respectively. Our findings are also in accordance with the results of researchers above. Although highest DM yield were obtained when the GKG was cut 180- days, the forage was of poor quality as indicated by its low CP and mineral content, and high lignin content. On the other hand, when the GKG was cut every 30- days the quality was excellent but DM yields were lower, it was often difficult to maintain a good stand, more frequent weeding was necessary, and cost of harvesting was higher. Other researchers (Campos et al., 2002; Wadi et al., 2004) have mentioned that yields of DM in tropic grasses are associated with infrequent cutting but CP content not. For this reason, a point where the intersection of quality with a satisfactory yield should be proposed. Generally, a 60- day harvest interval would seem to be a reasonable compromise between high yields and ease of management in the present study, however with a somewhat shorter interval can be used during growing season.

The ANOVA indicated that cell wall components (NDF and ADF) were affected by year-cutting interval interaction (Table 2). The favourable average NDF content (53.6%) was recorded at 30- day cutting interval in the second year, whereas the highest content (73.9%)

was in the first year at 180- day. Year effect was not significant on NDF. Similar trend was determined in ADF content as the growth period increased. The maximum ADF content (46.7%) was measured in 2015 at 30- day cutting interval, while minimum ADF (36.7%) was measured at 180- day in 2016. Year effect was also significant and average ADF content of first year (42.1%) was higher than the following year (41.1%).

NDF and ADF contents of GKG silage increased with delaying harvesting interval as expected because of the progressing maturation of the grass. The cell wall components reflect the potential intake of forage (NRC, 1978; Mbuthia, 2003; Zewdu, 2008). These components tended to increase with increasing cutting interval. At large, silage made at 60- day cutting interval performed better than the other cutting practices with regard to NDF and ADF contents in the study. Although silage made at 120- or 150- days cutting interval seems to be the same group at 60- days with regard to cell wall components, the reason was that they had second re-growths, which were relatively fresh. Silage made at once cut during season had greater NDF and ADF concentrations than the other cutting treatments. Many research workers (Wadi et al., 2004; Shen et al., 2012) emphasized that hay or silage made by wizened grass is not a good option to feed livestock due to the lower quality. On the other side, some researchers emphasized that if managed well (plant age, cutting height, etc.) fresh or ensilaged GKG would almost meet the nutritional needs of cattle (NRC, 1978; Geren and Kavut, 2015). There is limited information available regarding the cutting frequency or interval on the effect of metabolisable energy or cell wall component of GKG grown under Mediterranean climatic condition. Average NDF and ADF concentrations of GKG grown at different plant density in Mediterranean climate were reported to be 54.0 and 45.3% when cut once during growing season at 15 cm stubble height in Turkey, respectively (Geren and Kavut, 2015). Kukkonen (2009) stated that young GKG first harvested at a height of 90 to 120 cm tall had a CP level of 19.5% of DM, with 56% NDF and 34% ADF in Illinois, USA. These figures are comparable to some legumes forage crops. Nevertheless, later, harvest of third growth the crop at a height of 120 to 220 cm on November had CP of 10.5%, 66% NDF and 37% ADF. Shen et al. (2012) reported that cell wall component of king grass silage affected by different harvest time and wilting process with mean of 60% NDF and 30% ADF.

Conclusion

The results of the study testing the effect of six cutting frequency (30-, 60-, 90-, 120-, 150 and 180- day intervals) on the crop showed that based on DM yield, CP and cell wall contents, using 60- day intervals should be recommended in the regions with Mediterranean-type climates and in similar agro-ecologies of the country or other countries as well. Future experiments on GKG crop should be conducted at different locations with various agronomical treatments and especially cutting intervals (40-, 50-, 60- or 70- day) to be sure that results are relatively consistent over time. Additional research activities with rumen digestibility are also needed.

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A PRELIMINARY STUDY ON THE EFFECT OF DIFFERENT NITROGEN LEVELS ON THE YIELD AND SOME YIELD CHARACTERISTICS OF FODDER BEET (*Beta vulgaris* var. *rapacea*)

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Abstract

A pot study was conducted at Ege University, Faculty of Agriculture, Department of Field Crops, Izmir/Turkey, from October 2017 to May 2018, to investigate the effect of nitrogen levels (0, 30, 60, 90, 120, 150, 180 and 210 kg N ha^{-1}) on the yield and some yield traits of a fodder beet (*Beta vulgaris* var. *rapacea* Koch.) in the outdoor conditions. The experiment was arranged as a completely randomized block design with four replications. Leaf length and weight, tuber length-diameter-weight, tuber sugar content and dry matter content were studied in this study. Results shown that rates of nitrogen had a significant effect on the entire measured yield and the yield components of fodder beet. In the Mediterranean ecological conditions of Izmir, the best yield in fodder beet was obtained by application of 120 kg ha^{-1} of nitrogen.

Keywords: *Fodder beet, nitrogen level, livestock and tuber weight*

Introduction

Production of forage crops is very important for livestock production in the world. The animal feed obtained from natural grassland is not sufficient for animal feeding (Hazar and Velibeyoglu, 2018). Forage crops growing rate should be increased in the crops cultivation. Root of fodder beet is basically grown for animal feeding (Al- Jbawi et al., 2015). It is fresh forage, highly valuable for winter feeding, with a positive effect on the health status of the animals (Slavova et al., 2004). The tuber of fodder beet is used as a flavourant especially for straw in winter-feeding of animals. Fodder beet resembles sugar beet in terms of farming and mechanization.

Successful integration of fodder beet into the farm system, like any crop, requires careful consideration and good practice to achieve high yields, good utilization, and manage transition risks (Paska, 1994). Nitrogen is often the most limiting factor in crop production. Thus, application of nitrogen fertilizer results in higher yield and protein concentration in plant tissue is commonly increased (Perchlik and Tegeder, 2017). The protein content of fodder beet is low and beet protein is quite soluble (N effective degradability of 85%). Nitrogen supplementation is therefore necessary (Dulphy and Demarquilly, 2000). This research aims to study the effect of different nitrogen levels on fodder beet (*Beta vulgaris* var. *rapacea*) yield and some yield characteristics under the conditions of Mediterranean environment.

Material and Methods

This study was conducted as a pot study in outdoor conditions on the experimental area of Field Crops Department, Faculty of Agriculture, Ege University, Izmir, Turkey from October 2017 to May 2018 with typical Mediterranean climate characteristics. Some meteorological data from the experimental area in Bornova-Izmir and some characteristics of the experimental soil are presented in Table 1 and Table 2, respectively.

Table 1. Some meteorological data of experimental area in Bornova, Izmir, Turkey in 2017-18

	2017- 2018		Long Year Average	
	Temperature (ºC)	Precipitation (mm)	Temperature (ºC)	Precipitation (mm)
October	18.8	45.7	19.1	43.2
November	13.3	62.1	13.8	109.7
December	11.8	73.9	10.5	137.9
January	9.1	58	9.0	112.2
February	12.1	116.8	9.2	99.7
March	15.8	67.2	11.8	82.9
April	19.3	11.3	16.1	46.4
May	23.9	67.6	21.0	25.4
Total-Mean	15.5	502.6	13.8	657.4

Table 2. Some characteristics of the experimental soil

Sand (%)	80.2	CaCO3 (%)	0.82
Clay (%)	1.8	Organic material (%)	2.27
Silt (%)	18.0	Total N (%)	0.092
Texture	Loamy	P (ppm)	2.54
pH	5.83	K (ppm)	40
Total salt (%)	0.03	Ca (ppm)	1300

"Brigadier *Z*" cultivar of fodder beet (*Beta vulgaris* var. *rapacea*) from Germany was used as trial material. The seeds were sown in a plastic pots filled with 20 kg loamy experimental soil on 12th October, 2017. Eight rates of nitrogen (0, 30, 60, 90, 120, 150, 180 and 210 kg N ha^{-1}) were used as randomized complete block design with four replications. Half a dose of nitrogen fertiliser (urea) was applied before sowing, and the rest of nitrogen was applied at the end of February. All plots were fertilised using 50 kg ha^{-1} P_2O_5 (triple superphosphate) and 100 kg ha^{-1} K_2O (potassium sulphate) before sowing. There were no problems with pests and diseases during the experiment. Weeds were manually removed from the pots; no herbicide was used to control weeds.

The plants were harvested for tuber on 9th May 2018 by hand. Leaf lenght (cm); the distance between the head of the leaf to the beginning of the root was measured by ruler. Tuber length (tuberous root) (cm); the distance between the head of the root to the tip of the root was measured by ruler. Tuber diameter (cm); the most swollen part root was measured by caliper. Leaf weight; leaves taken from the harvested pots were weighed. Tuber weight (g plant^{-1}); roots taken from the harvested pots were weighed. Tuber sugar content (%); sugar content was evaluated by refractometer. Tuber dry matter (DM) content (%); 100 g samples taken from the root were dried 24 hour at 105°C and DM content was measured. The obtained data were statistically processed by analysis of variance (ANOVA) with the Statistical Analysis System (SAS, 1998). If ANOVA indicated differences between treatment means, a LSD test (0.01) was performed to separate them (Stell, 1997).

Results and Discussion

Data presented in Table 3 shows that nitrogen levels had a significant effect on the all of tested characteristics in the present study.

Table 3. Effect of different nitrogen levels on the yield and some yield characteristics of fodder beet

N levels	Leaf length (cm)	Tuber length (cm)	Tuber diameter (cm)	Leaf weight (g plant^{-1})	Tuber weight (g plant^{-1})	Tuber Sugar Content (%)	Tuber DM content (%)
N0	22.0 d	9.3 c	2.9 e	37.3 d	35.0 g	9.3 d	12.6 e
N30	25.2 d	12.7 bc	4.0 d	38.0 d	42.9 g	10.9 c	13.2 de
N60	28.6 c	13.3 bc	4.8 cd	40.8 d	59.4 f	11.1 c	13.5 cde
N90	29.6 bc	19.2 b	6.4 b	68.8 b	195.0 b	12.2 bc	15.0 bc
N120	34.4 a	40.9 a	13.9 a	94.3 a	230.9 a	12.6 ab	16.0 b
N150	31.8 abc	19.4 b	5.6 bc	66.3 b	116.3 d	13.2 ab	20.4 a
N180	32.8 ab	18.9 b	4.7 cd	63.6 b	153.4 c	13.7 a	14.3 cd
N210	33.1 a	19.0 b	4.5 d	53.4 c	97.4 e	13.8 a	14.0 cde
Mean	29.7	19.1	5.8	57.8	116.3	12.1	14.9
LSD (1%)	3.3	8.4	0.9	5.9	13.4	1.3	1.5

Results presented in Table 3 showed that all N levels significant increased the leaf length as compared with control (N0). N120 gave the highest leaf length (34.4 cm). In addition, there was no statistical difference among N120, N150, N180 and N210. The lowest leaf length (22.0 cm) was measured in N0. These findings indicate that application of nitrogen fertilizer up to 120 kg N ha^{-1} increased fodder beet leaf length. This is due to the fact that increased concentration of chlorophyll can increase the photosynthetic rates and that increment has a positive effect on the leaf expansion and the leaf length (Najm et al., 2010). The increases in leaf length compared to control were 50.3 and 35.3 for the 143 kg N ha^{-1} and 0 kg N ha^{-1}, respectively (Hussein and Siam, 2014). Tsialtas and Maslaris (2008) pointed out that nitrogen application had a significant effect on maximum leaf length for which the highest values were found for the highest N level in sugar beet (240 kg N ha^{-1}). This result is consistent with the present results.

The highest tuber length (40.9 cm) and diameter (13.9 cm) were obtained from N120 level and the lowest tuber length (9.3 cm) and diameter (2.9 cm) were obtained from N0 level in our research (Table 3). The increase in nitrogen levels caused an increase in tuber length and diameter. Turk (2010) reported that tuber length (10.7, 13.1, 14.1, 14.8) and diameter (9.5, 11.6, 12.5, 13.2) of fodder beet were statistically significant increased by increasing nitrogen level (0, 70, 150, 225 kg ha^{-1}), respectively. In other study, 0, 40, 60 and 80 kg ha^{-1} N fertilizers were given to sugar beet by Sulfab et al. (2017). Researchers found that increased nitrogen levels enhanced the tuber diameter as 8.9, 9.6, 10.3 and 11.2, respectively. Ozkose (2013) stated that fodder beet average values of tuber length 24.7 cm and tuber diameter 11.6 cm were obtained by application of 150 kg N ha^{-1}. Present results are similar to those researchers' findings.

The heaviest tuber (230.9 g plant^{-1}) and leaf (94.3 g plant^{-1}) weight were obtained from N120 level (Table 3) and the least tuber (35.0 g plant^{-1}) and leaf (37.3 g plant^{-1}) weights were obtained from N0 level in this study. Tuber and leaf weight increased with increasing nitrogen application. Hussein and Siam (2014) expressed that N application (0- 143 kg N ha^{-1}) with increased nitrogen levels significantly enhanced tuber weight and leaf weight of fodder beet plants (tuber weight: N0:717, N143:1849 g plant^{-1}; leaf weight: N0:181, N143:514 g plant^{-1}). Sulfab et al. (2017) found that tuber weight of sugar beet increased with increasing N

fertilization level from 0 (516.2 g plant^{-1}) to 80 (799.5 g plant^{-1}) kg N ha^{-1}. Albayrak and Yuksel (2010) indicated that tuber yield of fodder beet was significantly increased by increasing nitrogen fertilizer level. They found that maximum weight of tuber was obtained by application of 200 kg N ha^{-1} but there were no statistical difference between 150 (96 t ha^{-1}) and 200 (100 t ha^{-1}) kg ha^{-1} nitrogen fertilizer applications in terms of tuber weights. Being the essential constituent of proteins, nitrogen is involved in all the major processes of plant development and yield formation (Perchlik and Tegeder, 2017).

Numerically, the highest sugar content (13.8%) was recorded at N210 level, whereas the lowest sugar content (9.3%) was observed at N0 level (Table 3). Moreover, there were no statistical difference among N120, N150, N180 and N210. Many researchers emphasized that the sugar content of the beet decreased as the N application rate increased (Sarhan, 2012; Abdel-Motagally and Attia, 2009). The all data suggest that beet plants having a continuous and adequate supply of nitrogen may preferentially synthesize the citric acid cycle products and their amino acids counterparts, and thus produce less sucrose. Photosynthetic products may also be channeled preferentially into new growth at the expense of sucrose being synthesized and transported to the root (Tsialtas and Maslaris, 2008).

N levels had significant effect on the DM content of fodder beet (Table 3). Maximum DM content (20.4%) was recorded in N150, and the minimum DM content (12.6%) in N0. Increasing fertilization with nitrogen tends to increase cell volume and hence increased moisture content; thus, the DM content of forage tends to decline (Capstaff and Miller, 2018). In fact, a decrease in DM content was observed after N150 nitrogen level in this experiment. This finding is in line with those obtained by Khogali et al. (2011), who reported increase in DM content of fodder beet with increase in levels of N (0, 40, 80 and 120 kg N ha^{-1}). They found that the greatest DM content was obtained by application of 40 kg N ha^{-1} (13.7%), and DM content was decreased with 80 and 120 kg N ha^{-1} application.

Conclusions

These results were obtained from a single pot trial conducted on sandy type of soil using the cultivar Brigadier Z, application of the higher rates of N levels increased the above mentioned traits compared to the control treatment. It can be recommended from the experimental traits of fodder beet grown at the Mediterranean ecological conditions that maximizing tuber yield could be obtained by adding 120 kg N ha per hectare. However, these results need to be confirmed in field trials conducted in different areas, using a variety of fodder beet planted on various soils.

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A PRELIMINARY STUDY ON THE EFFECT OF DIFFERENT PHOSPHORUS LEVELS ON THE GRAIN YIELD AND SOME YIELD CHARACTERISTICS OF HORSE BEAN (*Vicia faba* var. *minor*)

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Abstract

Horse bean (*Vicia faba* var. *minor*) has potential as a source of nutrition for animal feed, and as a N_2 – fixing. It also plays an essential role in enhancing soil fertility. Phosphorus is major nutrient elements for grain legumes. Legume crops can be quite responsive to P fertilization, particularly; where soils are low in available of P. The application of optimum level of phosphorous has an important role in getting high grain yield of horse bean. This study was conducted to determine the effect of phosphorus levels on the grain yield and some yield parameters of horse bean (Jasny cv.). The experiment was carried out at Ege University, Faculty of Agriculture, Department of Field Crops, Izmir/Turkey, from October 2016 to June 2017 as a pot experiment grown outdoor. In the experiment, horse bean seeds were sown with different fertilization levels of phosphorus (0, 20, 40, 60, 80, 100, 120 and 140 kg P ha^{-1}). Rate of phosphorus had a significant effect on all of the measured traits and the grain yield of horse bean. Application of phosphorus at the rate of 100 kg ha^{-1} can be recommended for obtaining the maximum grain yield in horse bean under Mediterranean ecological conditions of Izmir.

Keywords: *Horse bean, phosphorus level, harvest index and grain yield*

Introduction

Worldwide, legumes are primarily used as dry seeds for both human and animal nutrition. Faba bean (*Vicia faba* L.) is a valuable legume rich in proteins and carbohydrate. In this context, it is interesting to highlight that the protein content in faba beans can exceed 30% (on dry basis) (Crepon et al., 2010). Horse bean (*Vicia faba* var. *minor*) has potential as a source of nutrition for animal feed, and as a N_2 – fixing. It also plays an essential role in enhancing soil fertility (Jensen et al., 2010).

Nitrogen (N) and phosphorus (P) are major nutrient elements for pulses like bean. Legume crops can be quite responsive to P fertilization, particularly, where soils are low in available P (Hamed, 2003). Phosphorus has a key role in the energy metabolism of all plant cells and for nitrogen fixation in legume crops (Schulze, 2004). Moreover, the application of optimum level of phosphorus has an important role in getting high grain yield of horse bean. In many countries in the world, there are different types of studies regarding horse bean grain yield and yield components response to P fertilization. Therefore, the presented investigation aimed to determine the effect of different phosphorus levels on horse bean (*Vicia faba* var. *minor*) grain yield and some yield characteristics under the Mediterranean environmental conditions.

Material and Methods

This study was established as a pot study in outdoor conditions on the experimental area of Field Crops Department, Faculty of Agriculture, Ege University, Izmir, Turkey from October

2016 to June 2017 with typical Mediterranean climate characteristics. Some meteorological data from the experimental area in Bornova-Izmir and some characteristics of the experimental soil are presented in Table 1 and Table 2, respectively.

Table 1. Some meteorological data of experimental area in Bornova, Izmir, Turkey in 2016-17

	2016- 2017			Long Year Average		
Months	Temperature (ºC)	Precipitation (mm)	Relative Humidity (%)	Temperature (ºC)	Precipitation (mm)	Relative Humidity (%)
October	19.2	1.4	45.7	19.1	43.2	64.0
November	14.0	100.6	62.1	13.8	109.7	68.9
December	6.8	15.2	73.9	10.5	137.9	71.7
January	6.3	237.6	73.9	9.0	112.2	70.1
February	10.4	55.6	69.0	9.2	99.7	68.1
March	13.3	72.2	71.0	11.8	82.9	66.1
April	16.6	15.7	59.3	16.1	46.4	62.9
May	21.7	27.0	57.7	21.0	25.4	59.6
June	26.5	1.8	56.2	26.0	7.5	52.9
Total-Mean	14.9	527.1	63.2	15.1	664.9	65.1

Table 2. Some characteristics of the experimental soil

Sand (%)	80.2	CaCO3 (%)	0.82
Clay (%)	1.8	Organic material (%)	1.27
Silt (%)	18.0	Total N (%)	0.092
Texture	Loamy	P (ppm)	1.14
pH	5.83	K (ppm)	40
Total salt (%)	0.03	Ca (ppm)	1450

Jasny cultivar of horse bean was used as test material. The seeds were sown in a plastic pots filled with 17 kg experimental soil on 25th October, 2016 at 2-3 cm depth. Eight rates of phosphorus (0, 20, 40, 60, 80, 100, 120 and 140 kg P ha^{-1}) were used as randomized complete block design with four replications. Nitrogen was added to all pots at a level of 20 kg N ha^{-1} and also potassium at a level of 100 kg ha^{-1}. Full doses of tested phosphorus (triple superphosphate) and K (as potassium sulphate) and rate of N (urea) were mixed with soil and supplied as a single application. Weeds were manually removed from the pots; no herbicide was used to control weeds.

The plants were harvested for grain (moisture: ~13%) on 16th June 2017 by hand sickle. Two horse bean plants were separated from each pot after harvesting to assay plant height and number of pods. Grain yield was weighed from each pot after harvesting. Biological yield was weighed by drying harvested plants. The grains were taken from each pot and 1000 seeds counted by hand and then weighed for thousand-grain weight.

The obtained data were statistically processed by analysis of variance (ANOVA) with the Statistical Analysis System (SAS, 1998). If ANOVA indicated differences between treatment means, a LSD (Least Significant Difference) test (0.01) was performed to separate them (Stell et al., 1997).

Results and Discussion

Data presented in Table 3 shows that phosphorus levels had a significant effect on the all of tested characteristics in the experiment. Results presented in Table 3 showed that all P levels significantly increased the height of plants as compared with control (P0). Maximum plant height (126 cm) was recorded in P120, and the minimum plant height (98 cm) in P0. Moreover, there were no statistical differences among P60, P80, P100, P120 and P140. As it

widely known, phosphorus increases the plant height by increasing cell division and elongation (Assuero et al., 2004). Turk and Tawaha (2002) found that plant height of faba bean increased with increasing P fertilization level (0, 17.5, 35, 52.5 kg P ha^{-1}) from 0 (62.3 cm) to 52.5 (77.0 cm) kg P ha^{-1}. Other researchers expressed that P application (0- 25 kg P ha^{-1}) on plant height of faba bean was not significant in spite of increased P levels (Nikfarjam and Aminpanah, 2015). Our findings partially confirmed those researcher's results.

Table 3. Effect of different phosphorus levels on the grain yield and some yield characteristics of horse bean

P levels	Plant height (cm)	Number of pod ($plant^{-1}$)	Biological yield (g $plant^{-1}$)	Grain yield (g $plant^{-1}$)	Harvest index (%)	Thousand grain weight (g)
P0	98 d	25.8 e	109 d	33.0 f	30.6 c	451 d
P20	109 cd	35.8 d	126 cd	50.5 e	40.2 b	590 c
P40	114 bc	38.5 cd	126 cd	59.3 d	47.3 ab	595 c
P60	116 abc	45.3 bc	143 bc	70.8 c	49.9 a	649 bc
P80	119 abc	51.0 ab	168 b	83.3 b	50.0 a	746 a
P100	125 ab	54.8 a	225 a	95.3 a	42.4 ab	716 ab
P120	126 a	54.0 ab	225 a	95.5 a	42.6 ab	658 bc
P140	125 ab	55.8 a	226 a	95.8 a	42.8 ab	613 c
Mean	117	45.1	168	72.9	43.2	627
LSD (1%)	10.8	9.2	32.2	8.5	9.3	78.7

ANOVA showed that the number of pods per plant was significantly influenced by the effects of P application (Table 3). The highest number of pods per plant was recorded (55.8) in P140 and the lowest number of pods per plant was (25.8) in P0. Nevertheless, there were no significant differences among P80, P100, P120 and P140. Pod number per plant increased with increasing P application, in current study. Previous studies have suggested that P increases flowering and fruit setting; thus, the number of pods per plant increased with the application of P. Many researchers emphasized that the number of pods per plant increased as the P application rate increased (Singh et al., 2011; Turk and Tawaha, 2002; Shakori and Sharifi, 2016). On the other hand, our results are not in line with Kubure et al. (2016). They found that faba bean did not respond to phosphorus application (0 and 46 kg P ha^{-1}) in terms of pod number $plant^{-1}$.

P levels had significant effect on the biological yield of horse bean (Table 3). The lowest average biological yield of horse bean (109 g $plant^{-1}$) was measured in P0 and the highest was (226 g $plant^{-1}$) in P140. Increasing P levels increased biological yield. Further, non-significant differences were observed among the levels of P100, P120 and P140 with regard to the biological yield. In addition, there were no significant differences between P20 and P40. Likewise, there were no difference between P60 and P80. Our results are in agreement with the result of Shakori and Sharifi (2016) who reported that maximum biological yield of faba bean (10.2 t ha^{-1}) was obtained by application of 100 kg P ha^{-1}, which was 40% higher as compared to control plot (0 kg P ha^{-1}). Similar results were recorded in another research about faba bean (Kubure et al., 2016). Results have shown that significantly greater biological yield has been obtained with the application of 46 kg P ha^{-1} (7.2 t ha^{-1}) than that obtained in control plots (5.6 t ha^{-1}). P can enhance photosynthetic energy transfer pathway because of efficient utilization of applied P fertilizer increased vegetative growth, which resulted for higher biomass production (Erkossa and Teklewold, 2009).

Increasing levels of phosphorus significantly increased yield of grain (Table 3). P140 gave highest grain yield (95.8 g $plant^{-1}$) which was followed by P120 and P100. Moreover, there were no statistical differences among P100, P120 and P140. The lowest grain yield (33.0 g $plant^{-1}$) was obtained in P0. These findings indicate up to 100 kg P ha^{-1}, horse bean grain yield

increased by application of phosphate fertilizer. According to Shakori and Sharifi (2016) results, highest (4.9 t ha^{-1}) and lowest (2.5 t ha^{-1}) values of grain yield in faba bean were obtained by application of 150 kg P ha^{-1} and in control treatments, respectively. Abou-Salama and Dawood (1994) found that increasing in phosphorus up to 90 kg ha^{-1} could increase yield of broad bean production. Based on results of 31 fertilizer trials (1967-1973) on faba bean, it was concluded that response to phosphorus was high, increasing P from 36 to 72 kg ha^{-1} increased yield by 9.8% and 15.7% over control (EIAR, 2011). These results are in line with our result.

There were significant differences among phosphorus levels in terms of harvest index (Table 3). The highest harvest index was recorded (50.0%) in P80 and the lowest harvest index was (30.6%) in P0. However, there were no significant differences among P40, P60, P80, P100, P120 and P140. Increasing P levels increased the harvest index up to P80 level, but after that, the harvest index decreased significantly. Similar results were expressed by Alipour et al. (2014) indicated that harvest index significantly affected by using phosphorus fertilizers in faba bean. On the contrary, Negasa et al. (2019) reported that harvest index of faba bean was non-significantly affected with increased P (0, 10, 20, 30 and 40 kg P ha^{-1}) levels. Kubure et al. (2016) stated that application of phosphorus (46 kg P ha^{-1}) tended to improve the harvest index (49%) of faba bean when compared with no P (0 kg P ha^{-1}) application (47%) though the difference was not significant. Our findings are not generally in accordance with those results.

Data regarding thousand grain weights are presented in Table 3. The minimum thousand-grain weight was produced (451 g) in P0, and P80 gave the highest thousand-grain weight (746 g) which was followed by P100. Also, there were no significant differences between P80 and P100. Increasing P levels increased the thousand grain weights up to P80 level, but after that, the thousand grain weights decreased, dramatically. Increased soluble P content increased the amount of phytin stored in the seeds. Phytin is an important compound for germination and seed growth with a significant contribution to seed size and weight (Nikfarjam and Aminpanah, 2015). In our study, however, phytin content of horse bean grain was not measured. Our results are in line with the findings of Shakori and Sharifi (2016) who also reported increase in 1000-grain weight of faba bean plants with increase in levels of P (0, 100 and 150 kg P ha^{-1}). They found that maximum values of thousand-grain weight were obtained by application of 100 kg P ha^{-1} (188 g). Hashemabadi (2013) reported that P levels (0, 40, 80 and 120 kg P ha^{-1}) not promote thousand-grain weights of faba bean plants significantly compared to control.

Conclusions

Results indicated that P levels significantly affected grain yield and other agricultural traits of horse bean. Application of the higher rates of P treatments increased the above mentioned characteristics comparing to the control treatment. Application of phosphorus at the rate of 100 kg ha^{-1} can be recommended for obtaining the highest grain yield in horse bean under Mediterranean ecological conditions of Izmir. However, this study should be repeated over both locations and years in order to give complete recommendation for practical application.

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PERFORMANCE OF SWEET SORGHUM (*Sorghum bicolor var. saccharatum*) AFFECTED BY NITROGEN MANAGEMENT IN SUSTAINABLE FORAGE CROPS CULTIVATION

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Abstract

Sweet sorghum (*Sorghum bicolor var. saccharatum*) is mainly planted for forage and sugar (ethanol) production. It is well adapted to sub-tropical and temperate regions, being highly biomass productive and water efficient. Recently, a great deal of research has been undertaken in almost every country of the world to explore sweet sorghum's biomass productivity and energy potential under various environmental conditions and cultural practices. A pot experiment was conducted to determine the effects of nitrogen levels on the forage yield and some quality components of sweet sorghum (*Sorghum bicolor var. saccharatum*). The experiment was carried out at the Bornova experimental fields of Field Crops Dept. of Agriculture Fac., Ege Univ., Turkey, during 2019-second crop growing season. The experiment was laid out in randomized complete block with four replicates. Seven nitrogen levels (0-50-100-150-200-250-300 kg N ha^{-1}) were tested on sweet sorghum cv Sugar-drip. Results indicated that the effects of N treatments on plant height, dry matter (DM) yield and cell wall components were significant. Moreover, application of the higher rates of N treatments increased the yield and yield components compared to the control treatment. The highest DM yield and acceptable content of cell wall components were obtained with the application of 200 kg ha^{-1} N in sweet sorghum under second crop production system.

Keywords: *Sweet sorghum, N level, forage yield*

Introduction

Sweet sorghum is a promising multi-purpose crop that has great potential for forage, grain or bioethanol production due to its wide adaptability, high sugar yield, and drought tolerance (Almodares et al., 2008). Thus development of sweet sorghum would play an important role in promoting the development of sustainable agricultural production, livestock husbandry (Fazaeli et al., 2006), renewable energy sources (biofuel) (Paterson et al., 2009).

Among the various inputs that improve the efficiency of a cultivar in realizing its potential, fertilizers, and nitrogen in particular, play a crucial role. Nitrogen deficiency decreased leaf area, chlorophyll content, and photosynthesis of sorghum plants that resulted in lower dry matter accumulation (Zhao et al., 2005). The crop also has great genetic variability and is good in N use efficiency. Application of fertilizers has a direct impact on crop productivity, and, nitrogen is one of the major nutrients that support crop growth and is the most responsive nutrient required by sorghum (Gardner et al., 1994)

The balance between the nutrient utilization and fertilizer amendment is desired in a production system. Determining the recovery efficiency and the N utilization in forage production is essential to improve the system, since, in situations that the amount of N applied exceeds plant absorption capacity to convert into forage production, and it can be lost by leaching, volatilization, or accumulating in plant tissues, reducing the DM conversion

efficiency (Freney, 2013). The purpose of this study was to determine the effects of different nitrogen levels performance of sweet sorghum in the sustainable forage crops production.

Material and Methods

A pot study was conducted in outdoor conditions on the experimental area of Field Crops Department, Faculty of Agriculture, Ege University, Izmir, Turkey during 2019-second crop growing season with typical Mediterranean climate conditions. Some meteorological data from the experimental area in Bornova-Izmir and some characteristics of the experimental soil are presented in Table 1 and Table 2, respectively.

Table 1. Some meteorological data of research area in Bornova, Izmir, Turkey in 2019

	2019		**Long Year Average**	
	Temperature (ºC)	Precipitation (mm)	Temperature (ºC)	Precipitation (mm)
August	29.8	0.0	27.9	12.7
September	24.6	31.7	23.9	18.0
October	21.2	4.0	19.1	27.7
November	16.9	41.0	13.8	31.5
Total-Mean	23.1	76.7	21.2	89.9

Table 2. Some characteristics of the experimental soil

Sand (%)	80.2	CaCO3 (%)	0.82
Clay (%)	1.8	Organic material (%)	2.27
Silt (%)	18.0	Total N (%)	0.092
Texture	Loamy	P (ppm)	2.54
pH	5.83	K (ppm)	40
Total salt (%)	0.03	Ca (ppm)	1300

Sugar-drip variety of sweet sorghum (Sorghum bicolor var. saccharatum) was used as test material. The seeds were sown in a plastic pots filled with 17 kg experimental soil on 2th August 2019, at 1 cm depth. Seven rates of nitrogen (0, 50, 100, 150, 200, 250 and 300 kg N ha^{-1}) were distributed in a completely randomized block design with four replicates. Half a dose of nitrogen fertiliser (urea), 80 kg ha^{-1} P_2O_5 (triple superphosphate) and 100 kg ha^{-1} K_2O (potassium sulphate) were applied with sowing, and the rest of nitrogen ((NH□)□SO□) was applied at beginning of stem elongation. Weed control was performed by manual hoeing only. No evident crop diseases or pests were detected.

The plants were harvested when the sweet sorghum reached milk dough stage of seeds. Harvested fresh crops were weighed and dried to a constant weight at 105ºC during 48 h and then DM yield (g $plant^{-1}$) was calculated. Plant height (cm); the plant was measured from the soil surface to the top level of the plant before harvest. Crude protein (CP) content (%) of grain was determined using the Kjeldahl method (N %) with a conversion factor of 6.25. Cell wall contents of samples as the neutral detergent fiber (NDF) (%) and acid detergent fiber (ADF) (%) were analyzed according to sequential detergent analysis method (Van Soest et al., 1991).

The obtained data were statistically processed by analysis of variance (ANOVA) with the Statistical Analysis System (SAS, 1998). If ANOVA indicated differences between treatment means, a LSD test (0.01) was performed to separate them (Stell, 1997).

Results and Discussion

Data presented in Table 3 shows that nitrogen levels had a significant effect on the all of tested characteristics in our study.

Table 3. Effects of different N levels on the yield and some quality components of sweet sorghum

N levels	Plant height (cm)	DM yield (g plant^{-1})	CP Content (%)	NDF (%)	ADF (%)
N0	145 c	33.1 d	5.8 e	46.4 a	39.3 a
N50	153 c	41.2 cd	6.4 d	44.8 b	38.7 a
N100	168 bc	70.4 b	7.8 c	44.6 b	38.0 b
N150	179 b	72.8 b	8.0 c	43.4 c	37.3 c
N200	195 a	84.9 a	9.1 b	41.3 d	36.0 d
N250	172 b	67.1 b	9.4 b	40.4 d	35.6 d
N300	158 c	45.4 c	10.7 a	40.2 d	35.2 e
Mean	**167**	**59.2**	**8.2**	**43.0**	**37.2**
LSD (1%)	11	8.2	0.4	0.9	0.6

The plant height of sweet sorghum was affected by nitrogen application (Table 3). The highest plant height (195 cm) was obtained from N200 level, whereas the lowest was 145 cm for N0 level. Increasing N levels increased the plant height up to P200 level, but after that, the plant height considerably decreased. Also, there was no statistical difference among N100, N150 and N250. These results are similar to those by Barik et al. (2017) who found that the plant height of sweet sorghum increased with increase in nitrogen application (N0:155.2 cm, N50:175.8 cm, N100:186 cm). Uchino et al. (2013) observed that the application of N fertilizer had significant positive effect on the plant height, especially at the rate between 90 and 120 kg N ha^{-1} in sweet sorghum plant. N is necessary element in building of tryptophan, which is used in the formation of auxin, later plays an important role in the elongation of plant, and increases the activation of meristem cell, so the plant height will be increased due to increasing cell division (Su et al., 2011).

The DM yield of sweet sorghum increased noticeably by increasing nitrogen application comparing to the control level (N0) (Table 3). The maximum DM yield (84.9 g plant^{-1}) was obtained from N200 level and the minimum DM yield (33.1 g plant^{-1}) was obtained from N0 level in this study. DM yield of sweet sorghum increased by increasing nitrogen treatments from 0 to 200 kg N ha^{-1} but later decreased at 250 kg N ha^{-1}. Further, there was no statistical difference among three N levels (N100, N150 and N250). Almodares et al. (2006) reported that N levels (0, 90 and 180 kg N ha^{-1}) promote DM yield (1653, 2367 and 2843 g m^{-2}) of sweet sorghum plants significantly comparing to control. Ayup et al. (1999) found that dry matter yield of sweet sorghum increased with increasing N fertilization level from 0 (8.1 t ha^{-1}) to 100 (13.3 ha^{-1}) kg N ha^{-1}. Our results for these parameters were in agreement with those of many other researchers (Patel et al., 1994; Uchino et al., 2013; Muhammed et al., 2002).

Results presented in Table 3 showed that all N levels significantly increased the CP content as compared with control (N0). The highest CP content (10.7%) was recorded at N300 level and the lowest CP content (5.8%) was recorded at N0 level. CP content increased with increasing nitrogen application in this research. As it widely known, sweet sorghum is by nature low in CP (Geren et al., 2013). CP content was increased significantly by increasing nitrogen level in forage sorghum at the 50% heading stage (N0:7.3%, N60:8.5%, N120:9.8%, respectively) (Sher et al., 2016). The increase in CP content with increasing in nitrogen fertilizer levels may be the result of enhancement in amino acid formation due to fertilization (Patel et al., 1994). The nitrogen application not only affects sorghum forage production but also improves its

quality from viewpoint of CP contents. The increase in CP content with nitrogen fertilization is in line with the findings of Ayub et al. (1999) and Almodares et al. (2009).
NDF and ADF contents were decreased with increasing N application (Table 3). Maximum NDF (46.4%) and ADF (39.3%) contents were recorded in N0, and the minimum NDF (40.2%) and ADF (35.2%) content in N300. Nirmal et al. (2016) stated that ADF content (49.3, 46.3, 43.9, 42.0 %) and NDF content (71.2, 68.6, 67.9, 66.5 %) of forage sorghum were significantly decreased by increasing N level rates (50% N, 75% N, 100 % N, 125% N of RDF [Recommended Dose of Fertiliser] ha^{-1}). In Quebec/Canada on a sandy loam, sweet sorghum NDF content decreased as N rates increased from 0 to 123 kg N ha^{-1} (Belanger et al., 2017). According to Restelatto et al. (2014) there was no difference in NDF and ADF contents in sorghum across N (210 and 215 kg N ha^{-1}) rates applied under the tropical conditions. And also, other researchers emphasized that ADF and NDF values did not change across N rates in forage sorghum (Lawton and Tech, 2020; Marsalisa et al., 2009). Our results partially match the findings of the researches mentioned above.

Conclusions

The outcome of this study shows that application of nitrogen fertilizer enhanced the some above-mentioned characteristics of sweet sorghum compared with the control. Application of nitrogen at the rate of 200 kg ha^{-1} can be suggested for obtaining the highest DM yield in sweet sorghum under second crop production system. Nitrogen effect on the yield and quality of sweet sorghum has been known to be dependent on the factors of climate, soil type and genotype, which also vary across seasons and locations. Because of these reasons, this study should be repeated both over locations and over years in order to give complete advice for farmer's application for the sustainable forage crops production.

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CALCIUM CARBONATE CONTENT IN THE SOIL OF NISAVA DISTRICT

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Abstract

Calcium is one of the most abundant elements in the lithosphere and the fifth most abundant element in the soil. It has a great impact on plants and affects the structure and pH value of the soil, and calcium deficiency in the soil causes an increase in soil acidity (decrease in soil pH). Plants need calcium for development. Calcium available to plants is formed after decomposition, most often caused by carbon dioxide (CO_2). The measure that is recommended in case of calcium deficiency in the soil is calcification. Carbonates can also be harmful; in the soil of a alkaline reaction and with a high content of lime, it reduces the absorption (availability) of phosphorus (P) and most microelements. The objective of this paper was to examine the abundance of soil with easily accessible forms of calcium ($CaCO_3$, %-calcium carbonate or calcite) in the territory of Nisava District (Southern and Eastern Serbia) in 2016, which covers an area of 2.729 km². The total number of samples was 2.013 from 357 agricultural farms. The $CaCO_3$ content in soil samples was determined with a calcimeter in accordance with the method of Scheibler. The obtained data were processed in the computer program IBM SPSS Statistic – trial version 20. The analysis showed that the soils in this area were insufficiently supplied with calcium carbonate. As many as 84.5% of the samples fall into the class of soil insufficiently provided with calcium, with a calcium carbonate percentage between 0-5%, while only 1.5% of the total number of samples can be classified as soil abundant in calcium carbonate.

Key words:*calcium, calcium carbonate, analysis, soil, Nis*

Introduction

Minerals play different roles in plant growth. It has been discovered that about 70 elements are present in plants, some of which are necessary, while others are considered useful elements. In order for an element to be considered important for a plant, certain conditions must be met: its deficiency prevents further growth of the plant; causes the plant chronic growth and development; the symptoms of its deficiency are more or less typical (Kastori and Maksimović, 2008). Macronutrients include carbon, oxygen, hydrogen, nitrogen, phosphorus, potassium, sulfur, calcium, magnesium. In addition to organic matter, the amount of nitrogen, phosphorus and potassium in the soil is most often tested in agriculture, so such tests are regularly performed in the Nisava District, Grčak D. *et al.* (2019a); Grčak D. *et al.* (2019b); Grčak D. *et al.*, (2018a); Grčak D., *et al.* (2018b). However, in addition to these elements, calcium is also one of the more significant elements from the agricultural aspect. Calcium is one of the most widespread elements in the lithosphere (3.6%), more precisely the fifth element in terms of presence in the soil (Phillips, 2018). Calcium is an alkaline earth metal that has the ability to build complex compounds, but does not affect the structure of organic matter. It affects the physical and chemical properties of the protoplasm, the stability of cell membranes and the activity of some enzymes. In addition, calcium significantly affects the

structure and pH of the soil. Calcium in the soil originates from primary silicon minerals and secondary calcium minerals, which are the parent material for soil formation. Since soils are formed on different substrates, a large difference in calcium content is evident in different soil types. Thus, sandy and peat soils contain about 0.25%, clay soils 3-6%, while limestone soils can have up to 30% CaO (Predić, 2011).

Primary and secondary minerals are the most important source of calcium in the soil. The calcium ion is very motile and susceptible to leaching in these minerals (Manojlović et al., 2014). The solubility and motility of calcium carbonate depend on the fineness of the particles, i.e. the contact area of $CaCO_3$ with H_2O and CO_2 (the larger contact area, the higher solubility). The state of high dispersion increases the solubility in water saturated with carbon dioxide. Potassium bicarbonate – $Ca(HCO_3)_2$ has a high solubility and is the most active calcium in the soil (Jelić, 2012).

The role of calcium in maintaining and increasing soil fertility is very important. The soil whose adsorption complex is saturated with calcium ions has good physical properties, high adsorption capacity and favorable conditions for microbiological processes, which affect the intensive mobilization of nutrients from soil reserves (N, S, P). Extremely acidic soils (pH < 4.5) practically do not have $CaCO_3$ (Whittinghill and Hobbie, 2012), so this should be taken into account during application of ameliorative measures and fertilization of these soils (Ubavić, 1996).

The content of calcium carbonate plays a significant role in the application of both organic and mineral fertilizers. It affects the effect of the applied fertilizers, both directly and indirectly, because its presence also affects the change of pH. Its presence in the soil has a special significance in the application of phosphorus fertilizers and some microelements. Calcium often limits the production of fruit species, causing a deficiency of some necessary microelements (iron, zinc, etc.). Excess calcium carbonate also has a detrimental effect on the availability of potassium in fruit trees, because it reduces its activity in the soil. In thestudy of Jaksić et al. (2017), a significant positive correlation was found between the total Mg content in the soil and the soil pH, as well as the $CaCO_3$ content.

Due to the unfavorable effects of a high $CaCO_3$ content in terms of the availability of certain microelements, it is necessary to pay attention during deep tillage. Soil layers with a high $CaCO_3$ content should not reach the active rhizosphere zone (Bogdanović et al., 1993). In soils that contain more calcium, the leaching of potassium is lower because calcium affects the deposition of colloids and prevents their dispersion, and thus the leaching of potassium. This means that calcification of acid soils prevents greater loss of potassium from the soil (Kastori et al., 2013).

Calcium salts are important for plant development, stimulates the development of root hairs and the growth of the entire root system. It is necessary for normal leaf growth. Soil pH varies depending on the season. During the summer, when microbiological processes are very frequent, it is lower, and during the winter, it is higher. The solubility of calcium, magnesium, iron and phosphate at pH values greater than 8 decreases sharply, while in an acidic environment the solubility increases (Stamenković, 2017).

Calcium in the soil is most often present in the form of calcite ($CaCO_3$) and dolomite ($CaMg(CO_3)_2$). Carbonates are less hard than other minerals, more soluble and easier to leach from the soil, therefore, they are more present in arid regions where leaching is minimal. The content of $CaCO_3$ in the soil changes slowly over time. The binding of metals to carbonates depends on the pH of the soil. Carbonates react easily with water, and bicarbonates (HCO_3^-) that can further react with soluble metals(eg lead or copper) are obtained. In carbonate soils, copper toxicity is significantly reduced by deposition of organic matter (Mihailović, 2015).

In acid soils, calcification is sometimes necessary, but it should be done very carefully, because excessive calcification can adversely affect the supply of plants with potassium, magnesium, zinc, manganese, iron, boron and copper (Varga, 2015).

Materials and methods

We monitored and analyzed 2.013 soil samples from the territory of Nisava District (2.729 km²) from 357 agricultural holdings. Our objective was to determine accessible calcium (in the form of calcium carbonate) in this territory during 2016. Statistical data processing was performed in the computer program IBM SPSS Statistic – trial version 20.
Soil samples were taken from a depth of 0-30 cm in field crops and vegetables, and 0-60 cm in orchards. An individual sample consisted of 20 to 25 individual samples (in lots up to 5 ha) from a depth of 0-30 cm in plots under field or vegetable crops or in plots where field or vegetable crops were planned to be grown, and from a depth of 0-60 cm, in plots under orchards or vineyards or in plots where orchards and vineyards were planned to be located.
The $CaCO_3$ content in the soil samples was determined quantitatively with a calcimeter in accordance with the method of Scheibler (Predić. 2011). A sample tube with HCl 1:3 is placed in a small bottle. Care must be taken to ensure that the acid does not come into contact with the soil. It is closed with a rubber stopper, allowing HCl to react with the soil. The CO_2 developed is read on the calcimeter, the pressure on the barometer, and the temperature on the thermometer.
The chemical reaction of decompose of calcium carbonate by HCl:
$CaCO_3 + 2HCl \rightarrow CaCl_2 + CO_2 + H_2O$
The amount of $CaCO_3$ is calculated by the formula:

$$CaCO_3\ \% = \frac{A \times B \times 2{,}274 \times 100}{C}$$

A = volume of developed CO_2 (ml)
B = weight 1 ccm CO_2 at a specific temperature and pressure (mm)
2,274 = CO_2 to $CaCO_3$ conversion factor
C = weight of soil sample (1 g)

Reagents (preparation 1:3 HCl): Measure 600 ml of distilled water and pour it into a 1-liter container. Gradually add 200 ml of concentrated HCl with constant stirring. Do all this in a digester (Manojlović et al., 1969; Komljenović and Kondić, 2011; Belić et al., 2014). After the analysis of all samples in the laboratory, the collected data were entered and processed in the SPSS software (trial version).

Results and Discussion

The obtained results were compared to the values presented in Table 1. to determine the soil supply with the readily available calcium forms and to clasiffy the soils that our samples were taken from. Calcium carbonate is important in the application of organic and mineral fertilizers, affects the reaction (pH) of the soil. Knowledge of the carbonate content is particularly important for perennial crops, due to the occurrence of chlorosis and inactivation of microelements. According to the carbonate content, soils are grouped into the following groups (Table 1).

Table 1. Soil classification as regards the readily available calcium-carbonate supply (Manojlović *et al.*, 1969)

Soil classification	**Content of % $CaCO_3$ in soil**
Carbonate-free soil	0%
Poorly supplied soil	0 – 5 %
Medium supplied soil	5 – 10 %
Well supplied soil	> 10%

The smallest plot in the study area is 1 are, and the largest is 35.45 hectares. The average plot size is 45 ares. The total area of all analyzed plots was 897.2 hectares of agricultural land. In order to determine the abundance of available calcium in the soil in the territory of the Nisava District in 2016, we performed an analysis of 2.013 soil samples.

Table 2. Soil classification as regards the quantity of the readily available calcium carbonates obtained in all studied samples from Nišava District, Southern and Eastern Serbia

Soil classification	***Number of samples (n)***	***Percentage (%)***
Carbonate-free soil (0%)	206	10.2
Poorly supplied soil (0-5%)	1.701	84.5
Medium supplied soil (5-10%)	75	3.7
Well supplied soil (>10%)	31	1.5
(Total)	**2.013**	**100.0**

The results of examining the calcium content in the soil in the Nisava District during 2016 can be seen in Table 2. Expressed in percent, 10.2% of the samples (206 samples in total or an area of 91.8 hectares) belonged to the soil class that did not contain calcium carbonate whatsoever. Most of the soils in our survey, 84.5% of the samples (1701 samples), were classified as the soil insufficiently abundant in calcium carbonate, the soil moderately abundant in calcium carbonate was found in 3.7% (75 samples), and we were able to classify only about 1.5% of the examined samples (31 samples) as the soil abundant in calcium carbonate. Based on the presented results we can conclude that the soils in the territory of the Nisava District are insufficiently abundant in calcium and that more attention should be paid to adding calcium to the soil. It would certainly be most desirable for all agricultural holdings to perform a soil analysis in order to be able to fertilize as efficiently and economically as possible for the agricultural crop they would like to grow (Vasin and Sekulić, 2005).

Unlike our study, it is possible that, irrigation with hard, saline water causes a slight deterioration of the chemical properties of the soil, increases soil pH, increases the calcium carbonate content, reduces humus, and increases microbiological activity and faster mineralization of organic matter (Mačkić, 2016).

Calcification of pseudogley soil, which is the main type of land in the studied areas, along with organic and mineral fertilizers, has influenced the reduction of soil acidity (Jerinić et al., 2015; Djurdjević, 2014).

According to the data for Vojvodina,(Vasin and Sekulić, 2005) the content of free $CaCO_3$ is at a much higher level than our studies show. Calcium carbonate was not detected in only 6.64% of the samples. This research was three years long and over 47.825 soil samples from the territory of the Autonomous Province of Vojvodina were analyzed. The content of CaCO3 in current study is lower in comparison to data for Vojvodina (Vasin and Sekulić. 2015) possible reasons for such a thing are insufficient or improper fertilization.

Conclusions

The content of calcium carbonate ($CaCO_3$) in the soil on the territory of Nisava District (Southern and Eastern Serbia) ranges from 0% (on an area of 91.8 hectares) to > 10% (on an area of 8.2 hectares). Otherwise, the most of studied soils (84,5%) are insufficiently supplied by CaCO3 (> 5%). Calcium deficiency in the soil, especially in the arable layer, occurs due occurs due to improper land use and due to insufficient calcium fertilization. Therefore, it is crucial that we maintain the calcium carbonate content optimal by proper fertilization, and it is also necessary to perform chemical analyzes of the content of elements in the soil before the exploitation of the land and to perform fertilization in accordance with the obtained results..

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THE PRESENCE OF FREE-LIVING NITROGEN-FIXERS IN SOILS OF STARA PLANINA MOUNTAIN IN SERBIA

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Abstract

The community structure and number of microorganisms are different in different soil types, and they represent the result of interactions between the soil type, plant species, localization of the microorganism in rhizosphere, anthropogenization and other factors. Certain microorganisms known for their ability to fix atmospheric nitrogen are also important for the development of healthy soil structure. Furthermore, they are important for the nitrogen input to soils in agroecosystem and represent economically attractive and ecologically acceptable means for reducing external inputs and improving soil quality. Soil samples were taken from pastures and meadows in the area of Stara Planina Mountain. According to its geographical and climatic conditions the area is characterized by typical hilly-mountainous regions with meadow and pasture systems for fodder production. The aim of this research was to examine quality of grasslands soil, so the parameters of soil fertility and number of free-living nitrogen-fixers from 55 locations were determined. The largest number of soil samples were characterized showed good amounts of soil organic matter, high nitrogen content and slightly acidic chemical reaction. Fjodorov agar was used for determining the number of free-living nitrogen-fixing microorganisms and their abundance ranged from 6.378 to 7.021 (log of number) per gram of absolutely dry soil.

Keywords: *free-living nitrogen-fixing microorganisms, soil, Stara Planina Mountain.*

Introduction

Soil microorganisms are important to many of the ecological processes that sustain life. They decompose soil organic matter, detoxify harmful chemicals, suppress disease organisms, provide nitrogen through fixation and produce substances that might stimulate plant growth. They represent an important link in the soil-plant system and contribute to the enhancement of soil fertility (Golić *et al.*, 2006; Emmerling *et al.*, 2002). From the aspect of plant production, the largest number of microorganisms in the soil is very useful to stimulate plant growth (Jarak *et al.*, 2005). In general, the level of microorganisms in the soil is positively correlated with the level of organic matter, which is mainly associated with the upper 30-40 cm of the soil.

Biological nitrogen fixation is one of the most important processes performed by soil microorganisms to convert nitrogen into the available form for the plants and improve the soil microbiological activities (Bano and Iqbal, 2016). The microorganisms that promote the nitrogen fixation play and important role, since this element is essential component of proteins, nucleic acids and other nitrogen compounds, and therefore of life for all living organisms (Döbereiner, 1997).

Oligonitrofiles, are fixers of atmospheric nitrogen for the supply of own needs and supplying plants with available nitrogen (Bogdanović, 1990). Large amounts of nitrogen mineral fertilizers have a negative effect on the microbiological activity of the soil, especially for the number of free living nitrogen fixers (Govedarica *et al.*, 1993). Asymbiotic nitrogen-fixers are

particularly sensitive to long-term contamination by high levels of heavy metals (Stajković Srbinović O. *et al.*, 2017). Microorganisms of this physiological group are supplied with small amounts of nitrogen from organic matter, and are partially able to use, in addition to organic nitrogen, also atmospheric nitrogen. The number of some groups of microorganisms and dehydrogenase activity are used as some of the indicators of the general microbiological activity and potential soil fertility (Milošević, 2008). The preservation of soil microbial diversity is crucial for a balanced agro-ecosystem, especially under increasing agricultural intensification (Marinković *et al.*, 2018).

The aim of this investigation was to examine the parameters of soil fertility and number of free-living nitrogen-fixers in the soil of pastures and meadows on area Stara Planina Mountain. This study provides new information to farmers and would contribute to implementation optimal models fertilization with organic and mineral fertilizers in order to increase the potential of the soil and also prevent degradation processes.

Material and Methods

Sampling of soil was carried out during September 2019 from the pastures and meadows located on the territory Municipitales of Pirot, Dimitrivgrad and Knjaževac in Serbia, area Stara planina Mountain (Tab. 1.). Soil samples from 55 locations were taken for the chemical and microbiological analysis aseptically at the depth of 0-25 cm. Soil sampling was carried out at three sites from different parts of each locality, then mixed and presented as one sample. The chemical properties of the soil were determined by standard methods in the chemical laboratory of the Institute for Forage Crops Kruševac.

The number of free-living nitrogen-fixers was determined on the agarized soil extract by indirect dilution method, by seeding the soil suspension from 10^{-5} dilutions. The number of grown colonies is calculated per 1 gram of absolutely dry soil (Jarak and Đurić, 2006). After incubation lasting five days at the temperature of 28°C, the number of grown colonies on the nutritive media was determined and calculated per 1g absolutely dry soil.

Results and Discussion

The quality and amount of soil organic matter, pH, metal concentrations, etc., have a pronounced influence on the dynamics of the microbial community structure and function in soils. The ecosystem functions of soil, to some extent, have a strong relationship with soil biogeochemical processes, which are linkages between biological, chemical, and geological processes (Dahlgren, 2006).

In this study sampled soils showed different acidity, but the greatest number was for acidic soils and slightly acidic chemical reaction (42 samples). The five soil samples had very strongly acidic chemical reaction, and only 8 of the analyzed soils showed a neutral reaction (Tab.1). According to Pislea and Sala (2012) chemical reaction of the soil is considered one of the most important characteristics of the soil environment. This factor has a strong influence on the growth of plants, the regime and biological availability of nutrients and the way that the ions from nutrients reach the root of the plant. The most of soil samples were characterized relatively well in terms of soil organic matter, high nitrogen content, and relatively well for easily soluble potassium and phosphorus. (Tab.1) The structure and functioning of microbial communities reflect interactions between a host of biotic and abiotic factors, among the most important of which is the quality of organic substrates available to it (Wardle and Giller, 1996). Garcia *et al.* (2017) reported that soil organic matter plays a key role in the development and functioning of terrestrial ecosystems, so both the quality and the quantity of SOM are quite important. According to Coleman (2011), roots and above-ground

plant growth are the most important sources of organic matter. Soils with higher SOM content have a more diverse and developed microbial population (Bo Liu *et al*., 2007).

Table 1. The chemical composition of studied soils

The municipality	GPS coordinates		pH		N (%)	P_2O_5 mg/100g	K_2O mg/100g	Humus (%)
	N	E	H_2O	KCl				
Dimitrovgrad	4753027	637032	6.10	5.00	0.200	8.80	13.90	3.00
Dimitrovgrad	4752629	637335	6.00	4.90	0.300	9.60	12.20	3.30
Dimitrovgrad	4752208	637141	6.02	5.10	0.246	23.23	49.61	279
Dimitrovgrad	4768123	649524	5.83	4.91	0.228	18.91	20.39	3.21
Dimitrovgrad	4767987	649509	5.85	4.77	0.298	31.30	30.19	3.12
Dimitrovgrad	4775905	654076	6.60	5.90	0.500	18.20	13.5	3.20
Dimitrovgrad	4775944	642725	6.60	5.78	0.346	56.60	21.26	3.05
Dimitrovgrad	4761008	644436	6.30	5.59	0.246	30.52	29.93	2.94
Dimitrovgrad	4760910	644583	6.35	5.78	0.322	31.31	39.00	2.97
Dimitrovgrad	4770655	643958	6.64	5.88	0.249	22.48	25.82	2.84
Dimitrovgrad	4763999	638758	6.28	5.35	0.242	27.98	39.19	2.91
Dimitrovgrad	4764041	638771	5.98	5.10	0.285	10.19	19.87	2.79
Dimitrovgrad	4764439	646886	6.55	5.88	0325	22.91	41.38	2.90
Dimitrovgrad	4776925	655072	6.78	5.90	0.600	47.41	17.38	2.91
Dimitrovgrad	4776821	655111	6.67	5.83	0.379	25.48	14.64	2.81
Dimitrovgrad	4775266	653552	7.50	6.51	0.386	80.15	264.96	2.87
Dimitrovgrad	4774594	646974	7.31	6.80	0.700	175.91	336.48	3.32
Dimitrovgrad	4772369	647566	5.12	4.19	0.258	15.45	15.79	2.73
Dimitrovgrad	4772174	647490	6.83	5.91	0.304	12.15	16.70	2.88
Dimitrovgrad	4772111	651934	5.25	4.45	0.271	27.13	42.70	2.86
Pirot	4795733	637094	6.94	6.01	0.684	83.48	245.28	2.97
Pirot	4777489	650633	7.21	6.30	0,366	18.83	31.42	3.13
Pirot	4771502	635371	6.35	5.56	0,304	16.52	38.93	3.03
Pirot	4785895	636063	5.45	4.79	0,461	8.00	11.38	2.97
Pirot	4792187	628134	7.07	6.53	0,376	34.18	52.25	3.25
Pirot	4783239	633916	7.30	6.85	0.333	29.30	50.69	2.99
Pirot	4787329	624486	7.24	6.51	0.479	28.40	40.51	3.29
Pirot	4778622	651128	6.57	5.77	0.420	19.90	39.00	3.36
Pirot	4791203	627460	6.35	5.87	0.205	18.33	31.92	3.09
Pirot	4787071	623462	5.83	4.94	0.219	15.41	19.80	3,08
Pirot	4789135	625232	6.98	6.09	0.269	10.90	17.09	3.27
Pirot	4791267	628490	6.40	5.79	0.353	11.18	23.30	3.26
Pirot	4791584	627370	7.35	6.91	0.329	21.83	36.62	3.14
Pirot	4778413	649716	6.40	5.79	0.550	6.70	16.06	3.25
Pirot	4783216	653636	7.00	6.24	0.235	23.13	32.54	3.26
Pirot	4774108	639218	6.88	5.94	0.282	10.15	41.36	3.36
Pirot	4775757	640328	6.34	5.70	0.380	22.44	62.35	3.25

Pirot	4773488	642309	7.37	6.72	0.322	20.25	28.80	3.13
Pirot	4788095	644961	6.94	6.16	0.411	28.31	63.79	2.80
Pirot	4788019	645019	5.82	4.91	0.397	19.98	19.78	2.76
Pirot	4773092	640705	7,29	6.44	0.363	33.30	39.31	3.06
Pirot	4780188	634053	7.50	6.88	0.301	9.0	28.46	2.77
Knjaževac	4802447	616796	6.90	6.10	0.200	10.80	26.20	2.30
Knjaževac	4816497	607614	6.30	5.50	0.300	13.40	41.50	3.10
Knjaževac	4816867	606770	6.50	5.70	0.200	12.80	19.00	3.00
Knjaževac	4814460	607971	6.00	5.10	0.300	18.10	39.30	3.20
Knjaževac	4799915	625523	5.50	4.80	0.300	14.90	21.70	2.90
Knjaževac	4799868	625773	5.30	4.40	0.400	13.20	25.60	2.70
Knjaževac	4833266	616265	5.20	4.40	0.300	19.60	32.70	3.10
Knjaževac	4816659	605938	5.10	4.10	0.200	15.50	25.90	2.90
Knjaževac	4832988	616983	5.50	4.60	0.300	12.10	45.40	2.80
Knjaževac	4807016	618087	5.90	5.00	0.400	22.80	58.90	3.00
Knjaževac	4808616	610002	5.90	4.90	0.200	12.20	49.60	3.00
Knjaževac	4799695	626939	6.30	5.60	0.200	18.30	37.10	2.90
Knjaževac	4806164	615639	6.30	5.40	0.200	5.30	19.70	2.50

The composition and function of the soil microbial communities varies greatly depending on their interaction with other abiotic and biotic factors, such as soil type, plant species, and other environmental variables (Stamenov *et al.*, 2016). Free nitrogen-fixing bacteria, that can fix atmospheric nitrogen, play an important role in maintaining soil fertility. In our study the number of free-living nitrogen-fixers in soil samples from varied from 6.378 to 7.021 (log of number) per gram of absolutely dry soil (Fig.1).

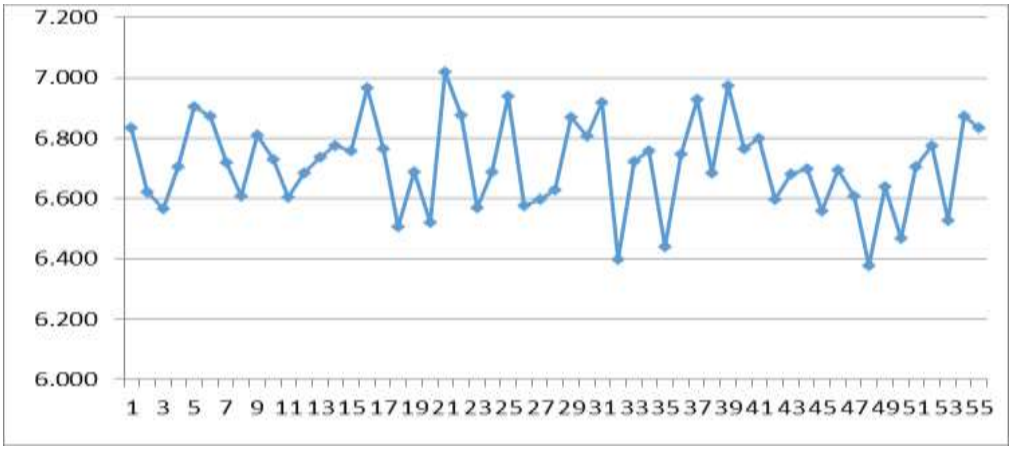


Fig. 1. The number of free nitrogen-fixing bacteria (log of number) in studied soils

Our results are in agreement with Rasulić et al. (2012). They show that the number of this microorganisms did not depend on a mode of utilization and their numerousness ranged from 0.67 to 164.00 10^5 g^{-1}. In soil type Chernozem Marinković *et al.* (1012) found at the depth of 0-20 cm the number of N_2-fixers from 80 to 516 x 10^6 g^{-1}. Also, Milošević *et al.* (2007) recorded high number of N-free living microorganisms - 7.19-7.63 (log. number of microorganisms) in soil of grasslands slightly acid with a high hummus content on Pešter. The indigenous microbial communities of each soil are unique for the particular soil, as they have

been shaped and evolved over time in accordance with the dynamics of the local habitat (Nazir *et al*., 2013).

Conclusions

Based on the results of this study, it can be concluded that tested soil samples have different chemical properties and the number of free nitrogen-fixing bacteria. Thanks to the activity of free living microorganisms the nitrogen content in the soil increases because of direct inputs of atmospheric nitrogen, so they have economic and ecological importance in agroecosystem.

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Ca:P RATIO AND CONTENT OF Ca AND P IN SOME ANNUAL LEGUMES-OAT MIXTURES DEPENDING ON SEEDING RATE AND STAGE OF GROWTH

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Abstract

Calcium and phosphorus are particularly important for animal health. The dietary levels of Ca and P should be balanced to increase their availability and utilization. The research was conducted at Institute for forage crops Kruševac in 2015-2016 to determine the effects of seeding rates in mixtures of pea : oat and common vetch : oat and cutting stages on the Ca and P content, as well as Ca : P ratio. The experiment was performed using five different mixture rates of pea and oat crops, five different mixture rates of common vetch and oat crops and three different cutting stages (beginning of pea and common vetch flowering – 10% of flowering, forming the first pods on 2/3 pea and common vetch plants and forming green seeds in 2/3 pods). Results of investigation showed that Ca content was the highest at the forming the first pods on 2/3 pea and common vetch plants, while phosphorus content increased from 2.70 to 3.40 g kg^{-1} with common vetch growth and development and from 2.57 to 3.10 g kg^{-1} with pea growth and development. Pure sown common vetch and pea were characterized by the highest average Ca and P content. Ca : P ratio decreased with plant growth and development in pea : oat mixtures, as well as in common vetch : oat mixtures. The highest Ca : P ratio was recorded in pure sown common vetch and pure sown pea, whereas the lowest Ca : P ratio was recorded in pure sown oat.

Keywords: *calcium, phosphorus, Ca : P ratio, pea, common vetch, oat*

Introduction

Mineral concentrations in forages vary greatly, and are affected by soil mineral level, soil pH, plant species, stage of forage maturity, and application of fertilizers or waste materials (Marković et al., 2019.). Forage mineral concentrations are of limited value in assessing mineral status of ruminants, because little is known regarding availability and factors affecting availability of minerals in ruminants fed forage diets (Spears, 2003). Minerals are required in livestock diets to meet nutritional and metabolic requirements. As changes occur in stages and levels of production, mineral requirements also change. To maximize animal productivity, adequate quantities of minerals need to be provided to livestock. If forage consumed does not meet the animals' requirement for specific minerals, mineral supplementation should be practiced (Greene et al., 1986). Forage crops are an important part oflivestock production, as they represent basic source of minerals in cattle nutrition (Suttle, 2010). The element composition of forage crops depends largely on soil type, plant species and its usage. Soil is the main source of macroelements and microelements for plants, however, only a small portion of their total content is plant available. A soil is considered deficient in a particular element if it can not provide that element in amounts sufficient for normal growth and development of plants or organisms (Hooda, 2010). The macroelement concentration generally changes with plant growth as the plant differs in photosynthetic capability and nutrition requirement at different growth stage.

In plant life, calcium and phosphorus are two important elements that play specific functions: calcium role is to give plants shape and consistency, to grow plants tissues and roots development, influences the cell membrane permeability and acts as an activator of some enzymes (Dordas et al., 2009; Khan et al., 2009; Hepler, 2005; Marnschner, 1995). Phosphorus is part of the cell structure composition, participates in several key processes including photosynthesis, respiration. It plays an important role in the processes of energy storage and transfer, in cell division and is a nutrients transporter through the plant cells (Dordas et al., 2009; Hepler, 2005; Marnschner, 1995). Calcium and phosphorus should be analyzed in combination because the dietary levels of Ca and P should be balanced to increase their availability and utilization (Albu et al., 2012)The Ca:P ratio of animal feeds should range from 1:1 to 2:1 (Miller et al., 1995). The National Research Council (NRC 1980) recommends a Ca:P ratio of 1.5-2:1. According to some authors, the optimal Ca:P ratio is 2:1 (Traba and Wolanski, 2003; Kumar and Soni, 2014). Diets with a Ca:P ratio higher then 2 can have a detrimental effect on animal health (Ayan et al., 2010). Grzegorczyk et al. (2017) cited Albu et al. (2012) indicated that inadequate Ca:P ratio can lower the availability, absorption and utilization of those elements. The objective of this study was to determine the content of Ca, P and Ca:P ratio in investigated mixtures of pea – oat and common vetch – oat depending on the stage of growth and seeding rate in the mixtures.

Materials and methods

Field pea and oat as well as common vetch and oat were grown in binary mixtures at the experimental field of the Institute for forage crops Kruševac, Serbia (21° 19' 35" E, 43° 34' 58" N). The study area was situated at altitude of 166 above sea level in South East Serbia. Soil type was with humus content of approximately 3.5% and a pH in H_2O 6.87, pH in 1N KCl 5.85; nitrogen content of 0.176%; Al-soluble P_2O_5 and K_2O 3.6 and 28.6 mg 100 g^{-1}, respectively. Experiment was established in autumn in 2015, on October the 20^{th}, and plant samples were taken in 2016. The experiment was designed with three replication according to a randomized complete block. All mixtures were sown on plots of 20 m^2.

The experiment was performed using three different cutting stages: A_1 – beginning of pea and common vetch flowering – 10% of flowering; A_2 – forming the first pods on 2/3 pea and common vetch plants and A_3 – forming green seeds in 2/3 pods of pea and common vetch plants. The pea-oat and common vetch-oat mixtures wee tested at five different mixture rates: B_1 – 100% pea + 0% oat and 100% common vetch + 0% oat; B_2 – 0% pea + 100% oat and 0% common vetch + 100% oat; B_3 – 25% pea + 75% oat and 25% common vetch + 75% oat; B_4 – 50% pea + 50% oat and 50% common vetch + 50% oat; B_5 – 75% pea + 25% oat and 75% common vetch + 25% oat. The ratio was formed according to the number of germinating grains of legumes and oat in the mixtures, where the germinating legumes grain per m^2 was taken as a reference for determining the ratio in the mixtures. The reference parameter for pea was 120 germinating grains per m^2, while 250 germinating grains per m^2 was taken for common vetch. Based on this values, the ratio of germinating grains in mixtures were formed.

Forage was cut and a sample of the whole plants was collected. Dry matter was determined by the drying out samples at 65° C and grinding and sieving them to 1 mm particle size. The concentrations of Ca was measured by atomic absorbance spectrophotometry – PERKIN ELMER 1100 B. Samples for Ca analysis were prepared with 1 g L^{-1} lanthanum. Phosphorus was measured colorimetrically, according to the ISO 6491 method using by HALO RB-10 Spectrophotometer. The data were processed by the analysis of variance in a randomized block design. The significance of differences between arithmetic means was tested by Fisher test ($p< 0.05$).

Results and discussion

The content of Ca, P and Ca:P ratio in investigated mixtures of pea – oat and common vetch – oat depending on the stage of growth and seeding rate in the mixtures were presented in the Table 1.

Table 1. The content of Ca, P and Ca:P ratio in common vetch-oat and pea-oat mixtures depending on the stage of growth and seeding rate in the mixtures

Treatments		Common vetch-oat mixtures			Pea – oat mixtures		
		Ca, g kg^{-1} DM	P, g kg^{-1} DM	Ca : P ratio	Ca, g kg^{-1} DM	P, g kg^{-1} DM	Ca : P ratio
A_1	B_1	7.0^{a}	2.7^{d}	2.59^{a}	6.7^{c}	2.6^{e}	2.58^{a}
	B_2	1.8^{f}	1.9^{e}	0.95^{f}	1.8^{h}	1.8^{g}	0.95^{f}
	B_3	3.8^{e}	2.7^{d}	1.41^{d}	3.4^{g}	2.5^{e}	1.34^{d}
	B_4	4.1^{d}	3.0^{c}	1.36^{d}	4.3^{e}	3.1^{b}	1.38^{d}
	B_5	4.7^{d}	3.1^{c}	1.51^{c}	4.6^{e}	2.8^{d}	1.65^{c}
	$\overline{X}$ **A_1**	**4.3^{B}**	**2.7^{B}**	**1.59^{A}**	**4.1^{B}**	**2.6^{C}**	**1.61^{A}**
A_2	B_1	7.5^{a}	+2.9^{c}	2.58^{a}	7.8^{b}	2.9^{c}	2.68^{a}
	B_2	1.9^{f}	3.1^{c}	0.61^{g}	1.8^{h}	2.6^{e}	0.69^{g}
	B_3	4.5^{d}	3.7^{b}	1.21^{e}	4.1^{f}	3.2^{b}	1.27^{d}
	B_4	5.6^{c}	3.0^{c}	1.86^{b}	5.4^{d}	2.9^{c}	1.87^{c}
	B_5	6.4^{b}	3.2^{c}	2.00^{b}	6.2^{c}	2.8^{d}	2.25^{b}
	$\overline{X}$ **A_2**	**5.2^{A}**	**3.2^{A}**	**1.62^{A}**	**5.0^{A}**	**2.9^{B}**	**1.76^{A}**
A_3	B_1	7.7^{a}	4.8^{a}	1.60^{c}	8.0^{a}	4.7^{a}	1.69^{c}
	B_2	1.9^{f}	2.9^{c}	0.65^{g}	1.8^{h}	2.4^{f}	0.76^{g}
	B_3	3.3^{e}	3.2^{c}	1.03^{f}	3.1^{g}	2.7^{d}	1.18^{e}
	B_4	3.6^{e}	3.2^{c}	1.12^{f}	3.4^{g}	2.9^{c}	1.16^{e}
	B_5	4.2^{d}	2.9^{c}	1.44^{d}	3.8^{f}	2.8^{d}	1.34^{d}
	$\overline{X}$ **A_3**	**4.1^{B}**	**3.4^{A}**	**1.20^{B}**	**4.0^{B}**	**3.1^{A}**	**1.29^{B}**
	$\overline{X}$ **B_1**	**7.4^{A}**	**3.5^{A}**	**2.11^{A}**	**7.5^{A}**	**3.4^{A}**	**2.20^{A}**
	$\overline{X}$ **B_2**	**1.9^{E}**	**2.7^{C}**	**0.70^{E}**	**1.8^{E}**	**2.3^{D}**	**0.78^{E}**
	$\overline{X}$ **B_3**	**3.9^{D}**	**3.2^{B}**	**1.21^{D}**	**3.6^{D}**	**2.8^{C}**	**1.26^{D}**
	$\overline{X}$ **B_4**	**4.4^{C}**	**3.1^{BC}**	**1.42^{C}**	**4.3^{C}**	**3.0^{B}**	**1.46^{C}**
	$\overline{X}$ **B_5**	**5.1^{B}**	**3.1^{BC}**	**1.64^{B}**	**4.9^{B}**	**2.8^{C}**	**1.75^{B}**

A_1 – beginning of pea and common vetch flowering – 10% of flowering; A_2 – forming the first pods on 2/3 pea and common vetch plants and A_3 – forming green seeds in 2/3 pods of pea and common vetch plants; B_1 – 100% pea + 0% oat and 100% common vetch + 0% oat; B_2 – 0% pea + 100% oat and 0% common vetch + 100% oat; B_3 – 25% pea + 75% oat and 25% common vetch + 75% oat; B_4 – 50% pea + 50% oat and 50% common vetch + 50% oat; B_5 – 75% pea + 25% oat and 75% common vetch + 25% oat; Values denoted by the different letter are significantly different according to Tukey's protected LSD values; $LSD_{0.05}$ – least significant difference at $P < 0.05$

Calcium concentrations differed significantly with plant growth and development of the analyzed common vetch-oat and pea-oat mixtures. The highest levels of Ca were noted at the second stage of plant development – at forming the first pods on 2/3 common vetch and pea plants, and the concentrations were 5.2 g kg^{-1} DM and 5.0 g kg^{-1} DM in common vetch-oat and pea-oat mixtures, respectively. Calcium content in the first and the third stage of plant development ranged from 4.3 to 4.1 g kg^{-1} DM in common vetch-oat mixtures and from 4.1 to 4.0 g kg^{-1} DM in pea-oat mixtures, and did not differ significantly. The content of phosphorus in the analyzed common vetch-oat mixtures increased from 2.7 to 3.4 g kg^{-1} DM with plant

growth and development. The same trend in phosphorus content was recorded in the pea-oat mixtures and phosphorus content increased from 2.6 to 3.1 g kg^{-1} DM with maturation. The analyzed common vetch-oat and pea-oat mixtures were characterized by optimal Ca:P ratio in the first and the second stage of development. In the common vetch-oat mixtures Ca:P ratio increased from 1.59 in the first stage of growth to 1.62 in the second development stage. After that, with plant growth and development from the second to the third stage of growth Ca:P ratio decreased to 1.20. In the pea-oat mixtures, Ca:P ratio increased from 1.61 in the first stage of growth to 1.76 in the second development stage, but after that decreased to 1.29 in the third stage of plant growth.

The highest content of Ca was determined in the pure sown common vetch (7.4 g kg^{-1} DM) and pure sown pea (7.5 g kg^{-1} DM), whereas pure sown oat was characterized by the smallest content of phosphorus. With increasing leguminous component in the mixtures content of Ca increased and ranged from 3.9 to 5.1 g kg^{-1} DM in common vetch-oat mixtures, and from 3.6 to 4.9 g kg^{-1} DM in pea-oat mixtures. The highest P content was also recorded in pure sown common vetch (3.5 g kg^{-1} DM) and pure sown pea (3.4 g kg^{-1} DM), but phosphorus concentration in mixtures changed very little with increasing leguminous component in the mixtures. Oat contained higher concentration of phosphorus than calcium. The highest Ca:P ratio was determined in pure sown common vetch (2.11) and pure sown pea (2.20), whereas, the lowest Ca:P ratio was recorded in pure sown oat (Table 1). With increasing leguminous component in the mixtures, Ca:P ratio increased, and ranged from 1.21 to 1.64 in common vetch-oat mixtures, and from 1.26 to 1.75 in pea-oat mixtures.

Researches confirmed that the best sources of calcium are leguminous plants. In general, leguminous plants are 2-3 times richer than grasses, whereas the calcium content is low in seeds and their products (Corbett et al., 2008; Hale and Olson, 2001; McDowell, 1992). Common vetch seeds are a rich source of minerals (Mao et al., 2015), although the content of individual minerals varies significantly among varieties (Uzun et al., 2011), ranging from 9.10 to 12.30 g kg^{-1} DM for potassium and from 2.30 to 5.07 g kg^{-1} DM for phosphorus. It is worth noting that the ratio of calcium to phosphorus is typically in the range of 0.24-0.73:1 (Mao et al., 2015), which is substantially lower than that recomended by NRC (2001). Also, many studies have shown that calcium level is higher in the early stages of vegetation and in higher proportions in the vegetative parts of plants, while leaves are richer than strains (Burlacu et al., 2002; Higginbotham et al., 2008). The same authors indicated that phosphorus content of feed decrease with increasing vegetation, therefore when the feedstuffs are harvested later, the dairy cows ration must be supplemented with phosphorus. Calcium is closely associated with phosphorus metabolism in the formation of bone, and a Ca:P ratio of 2:1 is usually recommended for ruminant diet. When Ca:P ratio is over 2.00, milk fever may be observed in animals. When Ca:P ratio is very low, calcium and phosphorus supplementation should be balanced in the diet of animals for their proper utilization in the animal system (Leng, 1990; McDowell, 1992).

Conclusions

According to the results obtained in this investigations we can conclude that common vetch-oat and pea-oat mixtures were sufficient in Ca content. The highest content of Ca was determined in the second development stage – at forming the first pods on 2/3 pea and common vetch plants. The highest P content was determined in the third stage of development - at forming green seeds on 2/3 pods of pea and common vetch plants. The optimal Ca:P ratios were determined in the first and second stage of development of common vetch-oat and pea-oat mixtures. Pure sown oat had inadequte content of Ca and P, as well as inadequate Ca:P ratio. Ca:P ratio increased with increasing common vetch and pea ratio in the mixtures.

The optimal Ca:P ratio for ruminant nutrition was determined in the mixtures which contained above 50% of leguminuos component in the mixtures. The optimal Ca:P ratio in investigated mixtures of pea-oat and common vetch-oat was recorded at the second development stage – at forming the first pods on 2/3 pea and common vetch plants.

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EFFECTS OF STORAGE LENGTH ON *ERYTHROSTEMON GILLIESII* (HOOK.) KLOTZSCH SEED GERMINATION

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Abstract

Erythrostemon gilliesii is evergreen shrub, decidous in colder climates growing 1-4 m tall. It is native in South America, but is now widely grown because of its decorative yellow flowers with long red stamens which bloom in summer. *E. gilliesii* is well adapted to dry conditions, growing easily in well drained soils in a full sun. Common propagation method of this species is by seed. The aim of this study was to investigate the effect of storage period of 10 years on germination and seed coat dormancy. After collection, part of the seeds was placed on germination, and the rest were stored in a room temperature, in dry conditions. After 10 years, 3 different treatments were conducted: soaking seeds in sulfuric acid at 96% for 15 minutes, mechanical scarification and soaking seed in water for 24 h (control). Germination of seeds immediately after collection (95%) was similar to germination in control after 10 years (98%). However, the germination energy after storage was considerably lower, only 23.5% compared to 82% recorded in a time of seed collection. The mechanical scarification and sulfuric acid treatment were not favourable for germination (76.5% and 85%, respectively), indicating that the additional pretreatments for breaking shallow seed coat dormancy were not required for *E. gilliesii*. The obtained results may also have ecological significance because *E. gilliesii* is capable of maintain high germination rate over a long period of time.

Key words: *Desert bird of paradise, seed storage period, sulfuric acid, coat dormancy, scarification.*

Introduction

Erythrostemon gilliesii Klotzsch (Fabaceae), commonly known by its synonym *Caesalpinia gilliesii* Hook. or *Poinciana gilliesii* Wall. ex Hook. is evergreen shrub, with slender, erect branches, decidous in colder climates. It is native in South America, but is now widely grown because of its decorative yellow flowers with long red stamens which bloom in summer. Bluish-green leaves are bipinnately compound, giving the fernlike appearance (Lewis, 1998; Cullen *et al.*, 2011). On its natural habitat, it can grow up to 8 m tall, but in cultivation is often 1-4 m tall. *E. gilliesii* is well adapted to dry conditions, growing easily in well drained soils in a full sun. The seed has been reported to have anti-cancer activity (Barboza *et al.*, 2009). In the invasive species databases (http://issg.org, http://www.iucngisd.org/gisd/, https://ec.europa.eu), *E. gilliesii* is not recorded as an invasive species, but it is recorded as naturalized in South Europe (including Italy, Spain and Greece).

Common propagation method of this species is by seed, and it is considered to have shallow seed coat dormancy (Baskin and Baskin, 2014; Kheloufi *et al.*, 2018). One of the important factors, both economical and biological, is a seed longevity, which can be defined as a period during which the seed preserves germination at cost-effective level, namely it can be used for commercial propagation (Ovuka *et al.*, 2010; Korekar *et al.*, 2013).

Taking into account that *E. gilliesii* is drought tolerant, low-maintenance, decorative, non-invasive species, it should be planted more often in Serbian green species. However, it has a

potential for naturalization. Besides other factors, presence of large and long-persisting seed banks and seed longevity can promote invasiveness of plant species (Moravcová *et al.*, 2006; Marchante *et al.*, 2010; Redwood *et al.*, 2019). Taking all into account, the seed longevity is an important factor both for commercial propagation and invasiveness potential of this species. Therefore, we decided to investigate the effect of the long storage period of 10 years on germination and seed coat dormancy.

Material and Methods

The fruits were collected in Pefkochori, Greece, in July. Upon bringing in the Laboratory for seed testing at the Faculty of Forestry, University of Belgrade (Serbia), dimensions and weight of fruits were measured as well as weight of seeds. These data were used for calculation of an extraction factor (the weight of cleaned seeds per given weight of fruits, expressed in percent), number of seeds in 1 kg and absolute weight of seeds (weight of 1000 seeds). Seven days after collection, 100 seeds (four replicates with 25 seeds each) were placed on germination without pretreatment, on the top of two layers of filter paper in the petri dishes. The remaining amount of seeds was kept in plastic bags placed in hermetically sealed metal boxes, at the room temperature, for 10 years. After 10 years of storage, the germination of seed was investigated using following pretreatments:

- mechanical scarification of seed coat
The seed coat was cut on side opposite from micropyle so the radicle was not damaged, using a sharp scalpel blade. After that, the seeds were soaked in water for 24 hours for imbibition.

- chemical scarification treatment
The seeds were soaked in a concentrated (95%) sulfuric acid (H_2SO_4) for 15 minutes, then carefully rinsed using a running water followed by rinsing in lime water solution in order to neutralize remaining of sulfuric acid. After this seeds were kept in distilled water for imbibition during 24 hours.

- Control treatment
The seeds were soaked for 24 hours to imbibe water.

Each treatment consisted of four replicates with 50 seeds each. The seeds were placed on the top of two layers of filter paper treated with 1% solution of the fungicide benomyl (commercial preparation "Benomil WP-50" manufactured by "Zorka" Šabac), in the petri dishes. Germination was carried out in long day conditions (light/dark period 16/8h), at the temperature of 20°C.
The number of germinated seeds was recorded daily during the period of 21 day. After that, the seeds were cut in order to determine the percentage of viable seeds which is necessary to calculate the real germination rate, as a percentage of sound (viable) seeds that germinate (Grbić *et al.*, 2010). The germination energy was recorded on 7th day. Obtained data were statistically analysed using the program Statgraphics Plus, Ver 2.1.

Results and Discussion

E. gilliesii pods collected in this experiment were averagely 74.92 mm long and 14.26 mm wide, containing 4.10 seeds (Table 1).

Table 1. Morphometric analysis of *E. gilliesii* pods and seeds

Parameter	Min	Max	$\bar{X}$
Length of pods (mm)	48	94	74.92
Width of pods (mm)	8	16	14.26
Thickness of pods (mm)	4	8	5.90
Weight of pods (g)	0.53	2.80	1.62
No of seeds per pod	1	8	4.10
Extraction factor (%)	12	43	32.5

Absolute weight of seeds is 135.5 g, and 1 kg contains 7375 seeds. Germination of *E. gilliesii* seeds was high, all non-germinated seeds were viable, and therefore germination rate and real germination rate were equal in all treatments (Table 2)

However, germination energy in a control was much lower compared to other treatments, only 23.5%. It can be observed that seven days after collection germination energy was relatively high (Table 2) and after 10 years, germination energy was also high when treatments for breaking seed coat dormancy were conducted, thus indicating that shallow seed coat dormancy developed during storage.

Table 2 Germination of collected seeds

Treatment	Germination rate	Germination energy	Real germination rate
Testing 7 days after collection	95.0^{a}	82.0^{a}	95.0^{a}
Control	98.0^{a}	23.5^{b}	98.0^{a}
H_2SO_4 treatment	85.0^{b}	81.5^{a}	85.0^{b}
mechanical scarification	76.5^{c}	75.5^{a}	76.5^{c}

Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

Long storage period didn't affect germination, and germination rate was even higher, reaching 98% after 10 years of storage (Table 2). However, higher germination rate was expected after H_2SO_4 treatment or mechanical scarification, but it was low compared to control. This can be explained by occurrence of infection caused by saprophytic fungi *Rhizopus nigricans* Ehrenb. which was recorded on some seeds after H_2SO_4 treatment and on large number of seeds after mechanical scarification. This fungi has been reported to decrease germination rate of infected seeds (Toba and Mohammed, 2014; Arya and Saxena, 1999; Rosińska *et al.*, 2013). The fungi occurred on seeds with damaged seed coat, it was not recorded in a control, and therefore soaking seeds in a warm water without damaging the seed coat should be investigated as a pre-germination treatment. Besides, better seed disinfection during germination of *E. gilliesii* should be considered.

Despite of difference between H_2SO_4 and control treatment, obtained germination rate was not low compared to the results obtained by other researchers. Namely, Kheloufi *et al.*, (2018) investigated germination of *E. gilliesii* 3 months after collection of seeds. They obtained best germination rate of seeds that were treated with H_2SO_4 for 30 minutes (95%), where in control germination was lower (82.5%), but difference obtained in their report was not statistically significant. Similarly, Galindez (2016) reported high germination rate of both mechanically scarified seeds (99.5%) and a control (96.9%).

The results obtained in our research showed that *E. gilliesii* seeds can be stored for a long time at a room temperature, preserving high germination rate. According to Genebank

Standards (1994) preferable conditions for longterm seed storage are 3–7% moisture content and temperature of -18°C. Therefore, our results can be used as a basis for a creation of lowcost protocol for *E. gilliesii* seed storage in genebanks.

Conclusions

Results obtained in our research showed that *E. gilliesii* seeds have no physical dormancy immediately after collection, and that there is no need to conduct any presowing treatment. The seeds can be stored in room temperature for 10 years, shallow seed coat dormancy develops during storage, and treatments for breaking dormancy can be conducted. However, very good results (98% germination rate) were obtained only by soaking seeds in water for 24 h. Presented results can be also used for establishing a procedure for *E. gilliesii* seed storage in genebanks.

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THE INFLUENCE OF TEMPERATURE AND SOIL TYPE ON GERMINATION SEED WHEAT

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Abstract

In order to achieve high wheat yields, it is necessary to determine the optimal plant composition, which is achieved through the use of quality seeds. Seed quality is affected by germination, purity and absolute seed mass. Seed germination is a variable trait. Seed germination is determined by standard methods under optimal laboratory conditions. Seed germination by standard methods is not real germination, because under natural conditions and under the influence of external factors and hereditary and non-hereditary seed properties, germination is much smaller. Seed germination and development of wheat under natural conditions takes place at much lower temperatures and in the presence of pathogenic soil microorganisms. For this reason, laboratory tests were carried out to determine the germination of seeds of two wheat varieties. The experiments were set on soil taken from two localities (East Sarajevo and Bijeljina). In addition to the standard seed germination method, we also took a test at temperatures of 2°C, 8°C and 12°C, respectively, at temperatures that are characteristic for our winter wheat production areas, for the period from October to December. After 10 days of seed germination in the growth chambers, the temperature was increased to 25°C and seed germination was observed for the next 6 days. The Russian variety had a higher percentage of seed germination compared to the domestic variety. Wheat seed sown in the soil of the humofluvisol type (Bijeljina) had higher germination compared to the seed sown in the soil of the alluvial type (East Sarajevo). The lowest germination had the seeds germinated at 2°C for 10 days, and then at 25°C for 10 days, and the biggest germination had the seed germinated at 12°C for 10 days, and then at 25°C.

Keywords: *Wheat, variety, germination, soil, temperatures.*

Introduction

Temperatures and uneven rainfall are the main factors in the variability of wheat yield in our agroecological area (Denčić et al., 2000). The overall life activity of plants is closely related to the temperature of the environment, with it being one of the most important factors for regulating the rate of development and flow of plant life cycle phases (McMaster, 1997; Jaćimović, 2012).

Under our agro-ecological conditions, the phenological germination phase takes place in the middle of October at an optimum temperature of 15-20ºC. The minimum temperature for this phase is 1-2ºC, the production optimum is 6-12ºC, and the maximum temperature that wheat can withstand during germination is 35ºC. At low temperatures, when the temperature is below 0°C, the seeds will not germinate, and at a minimum temperature below -20°C, it will freeze. Seed quality is a complex feature determined firstly by its germination. Determining germination of seeds by standard germination method (ISTA, 2003.) is carried out in ideal conditions so the results of the test can only apply for optimal field conditions (Siddique and Wright, 2004), and average germination often exceeds field growth (Hamman et al., 2002).

Seed germination and development of wheat under natural conditions takes place at much lower temperatures and in the presence of pathogenic soil microorganisms. With other constant factors, the degree of damage to seeds and young plants by pathogenic microorganisms in the soil depends on the rate of germination. At optimal humidity, the germination rate depends on the temperature.
In our agro-ecological conditions, during the sowing of winter wheat, unfavorable conditions for germination and sprouting occur, which is reflected in the uniformity of sprouting, the achievement of optimal plant assembly, and ultimately the yield itself. Therefore, the aim of this work was to determine the germination of seeds of domestic and Russian wheat varieties at soil temperatures corresponding to the sowing dates in certain production areas.

Material and Methods

The study was conducted in the laboratory of the Faculty of Agriculture in 2019, with the seeds of two winter wheat varieties (V_1 - selected in Republika Srpska and V_2 - selected in Russia) produced in 2018. The seeds were first sterilized in 96% alcohol and then washed several times in distilled water. Only clean and healthy seeds were used in the experiment. The experiment was set in four repetitions. We took soil samples from experimental parcels in East Sarajevo and Bijeljina After laboratory analysis it was established that alluvial soil (S_1) from East Sarajevo has pH_{H_2O} 6.63, it contains 3.62% humus, 0.23% N, and in 100 g^{-1} soil, it has 14.75 mg of soluble P_2O_5 and 15.59 mg of soluble K_2O, while humofluvisol (S_2) from Bijeljina had pH_{H_2O} 7.16, containing 4.12% humus, 0.27% N and 100 g^{-1} of the soil had a 40 mg soluble P_2O_5 and 36.41 mg of soluble K_2O. The sowing was done in plastic pots to a depth of 3 cm with 100 seeds in the pot. After sowing, the pots were kept for 10 days in chambers at temperatures of T_1 - 2°C, T_2 - 8°C, T_3 - 12°C. These temperatures were applied because average monthly soil temperatures at a depth of 2 to 5 cm in our production areas range from 12°C (October) to 2°C (December). After 10 days, the pots were transferred from the chambers to the growth chamber at a temperature of 25°C for 6 days. In addition to these experiments, a new experiment was set up, using a standard method for determining seed germination in Petri dishes on filter paper and at a temperature of 25°C. Germination was monitored during the experiment by counting germinated seeds (after 10 days) from each pot as a percentage of the total number of seeds, and then germinated seeds were counted every 2 days (D_1 - tenth day, D_2 - twelfth day, D_3 - fourteenth day, D_4 - sixteenth day).
The obtained results were analyzed by the two factorial reflection variance analyze (locality, absorbent), and the individual differences tested by the LSD test, using the SAS / STAT program (SAS Institute, 2000).

Results and Discussion

After ten days (Table 1), the seed germinated in the chamber at 2°C did not start germinating, while the average germination of seeds at 8°C was 41.88%, at 12°C 87% of the seeds germinated, and by the standard method of germination (25°C) germinated 93.88% of the seeds (Figures 7 and 8). The standard germination test is a good indicator of seed quality and viability, which can predict field emergence if soil conditions are near ideal (Durrant and Gummerson 1990).
The average germination of wheat seeds is 55.69%. Highly significant differences were found in the varieties, soil types and temperatures tested. Highly significant differences were found for the interaction of varieties x temperature, soil types x temperature and significant for the interaction of varieties x soil types x temperature (Figures 1, 2, 3, 4, 5 and 6).

The average germination of the V_2 variety is 64.34% and is highly significantly higher compared to the V_1 variety (47.03%). Seed germination depending on the soil type ranged from 52.31% (alluvial soil) to 59.06% (humifluvisol). The differences found are highly significant, as are the differences in the number of germinated seeds, depending on the temperature in the chambers in which the seed was germinated. Low temperatures, as well as other stress factors, cause the formation of reactive compounds in plants, resulting in damage to plant membranes and macromolecules. The availability of water as well as its movement is very important in the process of seed germination, initial root growth and elongation of cotyledons. These processes are greatly influenced by the chemical potential of the soil, texture and contact area of the seed and soil (Đurđić et al., 2016).

Table 1. Percentages of seed germination of 2 wheat varieties in soil types at Cold-test temperatures 2, 8 and 12°C during 10 days, compared with standard method (cca 25°C on sterile ground)

		Cold-test temperatures (°C) 10 days after sowing			Standard. Germination test (cca 25°C) (T_4)	Average
Variety (V)	Soil types (S)	2 (T_1)	8 (T_2)	12 (T_3)		
V_1	Alluvial (S_1)	0.00	5.25	80.50	90.25	44.00
	Humofluvisol (S_2)	0.00	23.25	86.75	90.25	50.06
	Average V_1	0.00	14.25	83.62	90.25	47.03
V_2	Alluvial (S_1)	0.00	51.25	93.75	97.50	60.62
	Humofluvisol (S_2)	0.00	87.75	87.00	97.50	68.06
	Average V_1	0.00	69.50	90.38	97.50	64.34
Average alluvial (S_1)		0.00	28.25	87.12	93.88	52.31
Average humofluvisol (S_2)		0.00	55.50	86.88	93.88	59.06
Average		0.00	41.88	87.00	93.88	55.69

	V	S	T	VxS	VxS	SxT	VxSxT
F	**	**	**	ns	**	**	*
$LSD_{0,05}$	3.224	3.224	4.559	4.559	6.448	6.448	9.119
$LSD_{0,01}$	4.305	4.305		6.089		8.611	
			6.089		8.611		12.177

*Mean values designated with the same lowercase letter were not significantly different at the 95% level according to the LSD test ** F-test significant at 0.01; * F-test significant at the 0.05 level; ns non-significant*



Figure 1. The effect of interaction $V_1xS_1xT_3$ on germination



Figure 2. The effect of interaction $V_2xS_2xT_3$ on germination

After 16 days of germination of wheat seeds, the average germination rate was 71.76% (Table 2). Highly significant differences were found for variety, soil types, the days of leaning and he

temperature at which the seed was germinated, as well as interactions of variety x soil types, variety x temperature, soil types x temperature, days x temperature, variety x soil types x temperature (Figure 9), variety x days x temperature (Figure 10), soil types x days x temperature (Figure 11). Variety V_2 (79.50%) had significantly higher germination compared to variety V_1 (64.02%). Highly significant differences in the number of seedlings were determined depending on the type of soil in which the seed was sown and ranged from 69.10% (S_1) to 74.42% (S_2). The number of germinated seeds, depending on the number of days of seed germination, ranged from 42.02% (D_1) to 82.38% (D_4). Highly significant differences between variant D_1 and other variants were found, as well as significant differences between variant D_2 and variants D_3 and D_4, while other established differences were not statistically significant.



Figure 3. The effect of interaction $V_2xS_1xT_3$ on germination



Figure 4. The effect of interaction $V_2xS_2xT_2$ on germination



Figure 5. The effect of interaction $V_2xS_1xT_2$ on germination



Figure 6. The effect of interaction $V_1xS_2xT_2$ on germination



Figure 7. The effect of interaction V_2xT_4 on germination



Figure 8. The effect of interaction V_1xT_4 on germination

Seeds germinated at 2°C for 10 days and then germinated at 25°C for 6 days had a germination rate of 49.84%, seeds germinated at 8°C for 10 days and then germinated at 25°C for 6 days, had a germination rate of 76.42%, and the seeds germinated at 12°C for 10 days and then germinated at 25°C for 6 days had a germination rate of 89.03%.

Highly significant differences were found in the number of germinated seeds in dependence of temperature in the seed growth chambers. The influence of low temperatures in the cold test did not have a negative effect on seeds living capability, which was established in previous tests (Vujaković et al., 2008) that leads to conclusion that physiological damage of

the seeds can be caused by extended and inadequate storage as well as by damage caused by drought or frost. The speed at which the seeds germinate can depend on genetic differences in seeds size, seedlings trait and chemical composition. After 10 days of germination, seeds of variety V_1 germinated with 32.43%, and varieties of V_2 with 52.37%, after 12 days germinated 72.59% of seeds of variety V_1, or 86.52% of variety V_2, after 14 days germinated 72.59% of seeds of variety V_1, or 89.20 % of variety V_2, after 16 days, 75.47% of seed of variety V_1 germinated, or 89.28% of variety V_2. With increasing treatment length, there was no significant increase in seed germination, which is in agreement with the results obtained by Samarah (2006) and Vukojevic et al. (2011) in their research. Germination ranged from 38.36% to 85.92% in the V_1 variety, depending on the temperature at which the seed was kept in the growth chamber, and from 61.31% to 92.16% in the V_2 variety. The tested varieties had a higher percent of germinated seeds was sown in humofluvisol (S_2) compared to alluvial soil (S_1).

Table 2. Percent emergence of winter wheat seed at temperatures 2, 8 and 12°C during 10 days after planting, and subsequently at cca 25°C. Mean for 2 varieties.

Variety (V)			V_1			V_2			VxDxT
Days after sowing	Temperatures (T)		VxSxDxT Soil types (S)		VxDxT	VxSxDxT Soil types (S)		VxDxT	DxT
			S_1	S_2		S_1	S_2		
D_1 (10 days)	T_1 (2°C)		0.00	0.00	0.00	0.00	0.00	0.00	0.00
	T_2 (8°C)		5.25	23.25	14.41	51.25	87.75	69.38	41.55
	T_3 (12°C)		80.50	86.75	84.32	94.21	87.00	89.60	86.39
VxSxD	VxD		28.05	36.13	32.43	47.62	57.74	52.37	42.02
D_2 (12 days)	T_1 (2°C)		46.00	46.75	46.20	71.25	85.75	78.71	62.76
	T_2 (8°C)		86.25	84.75	85.33	80.25	95.75	88.21	87.07
	T_3 (12°C)		84.25	88.75	86.86	95.67	90.25	92.58	90.32
VxSxD	VxD		71.97	73.22	72.59	82.61	90.41	86.52	79.86
D_3 (14 days)	T_1 (2°C)		51.50	53.50	52.14	77.50	87.50	82.88	67.51
	T_2 (8°C)		86.50	85.00	85.39	84.25	96.75	90.88	88.13
	T_3 (12°C)		84.50	89.00	86.92	96.75	91.25	93.79	90.36
VxSxD	VxD		74.15	75.82	74.80	86.18	91.85	89.20	82.00
D_4 (16 days)	T_1 (2°C)		52.25	56.75	54.14	78.00	87.50	83.13	68.63
	T_2 (8°C)		86.50	85.00	85.39	84.25	96.75	90.88	88.13
	T_3 (12°C)		84.50	89.00	86.92	96.75	91.25	93.79	90.36
VxSxD	VxD		74.40	76.90	75.47	86.35	91.85	89.28	82.38
VxS			62.53	65.91	64.02	75.28	83.32	79.50	**71.76**
VxT VxSxT	T_1 (2°C)		37.83	39.64	38.36	56.28	65.55	61.31	49.84
	T_2 (8°C)		66.51	69.89	67.83	74.59	94.61	84.99	76.42
	T_3 (12°C)		83.30	88.23	85.92	94.93	89.77	92.16	89.03
SxDxT SxD	0.00	27.70	85.95	37.06	0.00	55.30	86.99	46.88	
	59.13	83.76	90.23	77.60	66.36	90.36	90.44	82.11	
	64.89	85.76	90.44	80.36	70.12	90.50	90.29	83.64	
	65.51	85.76	90.44	80.57	71.75	90.50	90.29	84.18	S
SxD	47.45	70.95	88.91	69.10	52.22	81.88	89.15	74.42	
F	V **	S **	D **	T **	VxS **	VxD ns	VxT **	SxD ns	SxT **

$LSD_{0,05}$	1.867	1.867	2.641	2.287	2.641	3.738	3.234	3.738	3.234
$LSD_{0,01}$	2.466	2.466	3.489	3.021	3.488	4.937	4.272	4.937	4.272
	DxT	VxSxD	VxSxT	VxDxT	SxDxT	VxSxDxT			
F	**	ns	**	**	**	ns			
$LSD_{0,05}$	4.583	5.300	4.574	6.520	6.520	9.448			
$LSD_{0,01}$	6.053	7.000	6.041	8.611	8.611	12.479			

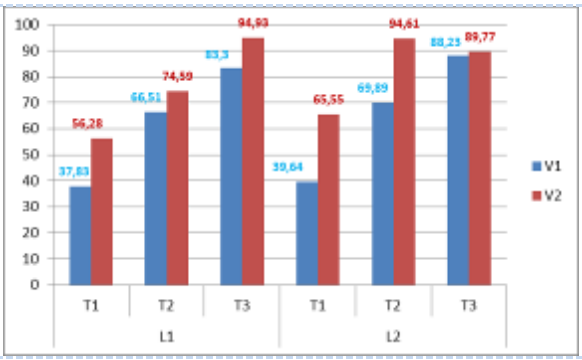


Figure 9. The effect of interaction VxSxT on germination



Figure 10. The effect of interaction VxDxT on germination

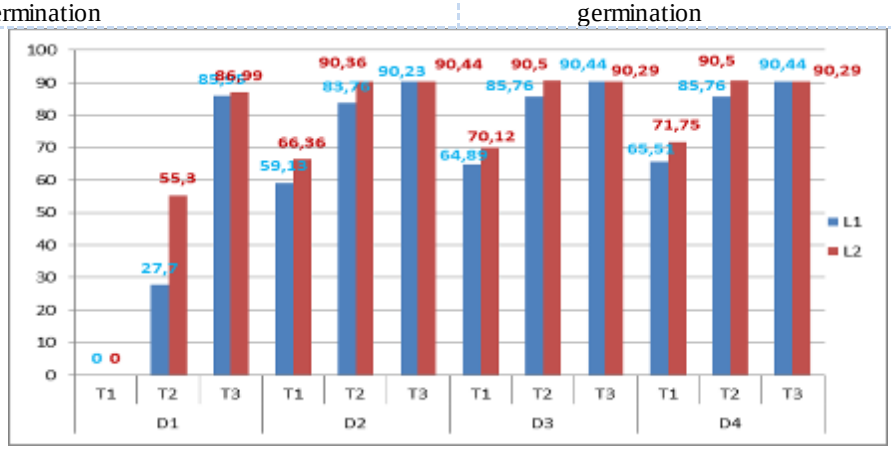


Figure 11. The effect of interaction SxDxT on germination

Conclusion

Based on laboratory studies of the influence of the variety, soil types, temperature at which the seed was germinated and length of germination (days) we can conclude:

- Germination of wheat seeds, determined by standard methods, was the highest, but this method does not give the true useful value of the seeds because such germination cannot be achieved under natural conditions;
- The Russian variety had a higher percentage of seed germination compared to the domestic variety;
- Wheat seed sown into the soil of the humofluvisol type (Bijeljina) had higher germination compared to the seed sown into the soil of the alluvial type (East Sarajevo);
- The highest seed germination was found in seeds that had germinated for 16 days;
- The lowest germination is for seeds that have been germinated at 2°C for 10 days and then at 25°C, and the highest germination for seeds that has been germinated for 10 days at 12°C and then at 25°C.

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EFFECT OF COLOR AND FLUX OF LED LIGHT ON GROWTH AND GRAFTING EFFICIENCY OF CUCUMBER SEEDLINGS

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Abstract

The aim of this experimental work is to study the influence of different light colors (white, red, blue, red + blue and green) and photosynthetic photon flux (25, 50 and 75 μmol m^{-2} s^{-1}) on growth of the grafted cucumber seedlings. LED lamps were used as source of light with different colors. The percentage of grafting efficiency (graft-take) was also calculated. The shoot length ranged from 7.97 to 9.66 cm, the stem diameter ranged from 4.007 to 4.785 mm, the leaves number ranged from 1.77 to 2.27 leaf/plant for, the leaf area ranged from 46.36 to 58.34 cm^2, the chlorophyll content increased from 22.80 to 32.67, the shoot dry weight ranged from 366.19 to 410.39 mg, and the grafting efficiency ranged from 72.83 to 89.52 % for different light colors and photosynthetic photon fluxes (PPF).

Keywords: *grafting, LED light, photosynthetic photon flux, grafting efficiency, cucumber.*

Introduction

The total cucumber crop production in Egypt was about 520 thousand tons in 2016 (FAOStat, 2018). Cucumber is one of the most important economic vegetable crops, practiced by the Egyptian farmers. Grafting is a horticultural technique whereby tissues from one plant are joined with another to obtain a combination of rootstock and scion genotypes that are more desirable than those contained in a single plant. Vegetable grafting is considered a sustainable pest and disease management practice that can improve fruit yields and quality as well as reduce environmental impacts with fewer chemical fumigants applied than in a non-grafted cropping system. Moreover, according to Lee *et al.* (2010), Louws *et al.* (2010), Schwarz *et al.* (2010) and Savvas *et al.* (2010), grafted vegetables can exhibit excellent tolerance to both biotic stresses (e.g., soil-borne pathogens, foliar pathogens, arthropods and weeds) and abiotic stresses (e.g., low/high temperature, drought/flooding, heavy metal and nutrition). Even though grafting has been practiced in fruit trees for thousands of years, vegetable grafting has only recently been adopted on a commercial scale (Sakata *et al.*, 2007). After grafting, it is important to control the environmental conditions surround grafted seedlings for the robust joining of scion and rootstock. Usually the shading means and plastic films are used to prevent grafted seedlings from wilting by excessive transpiration and to promote the healing of grafted plants in greenhouse or tunnel. It is quite difficult to optimally control the environmental conditions for healing and acclimation of grafted seedlings under natural light. Therefore, the growers or managers rely on their empirical knowledge for healing and acclimation of grafted seedlings (Kim, 2000, Jang *et al.*, 2011 and Khater, 2017). The light plays an important role in photosynthesis, growth and morphogenesis of plants that depends on the light wavelength (Avercheva, 2009) in addition to being one of the variables that affect the concentration of phytochemicals on them (Pérez *et al.*, 2008). For this reason, the artificial lighting systems used for crops in controlled environment (Kozai, 2007), in addition affect the cost and nutritional quality of greenhouse vegetables (Tamulaitis *et al.*, 2005). The LED is a solid state light source, durable and has narrow wavelength band (Gupta and Jatothu, 2013 and Xu *et al.*, 2012) that can be used in a variety of horticultural applications (Stutte, 2009).

These sources can be implemented in dynamic lighting to control growth, development, production and physiological responses of plants (Folta and Childers, 2008 and Lefsrud *et al.*, 2008). Light property regulates the plant development from germination to flowering induction and fruit stage (Jiao *et al.*, 2007). LEDs are the new fourth-generation light sources with good spectral characteristics and spectral width, and can be assembled to prove light quality which plants need (Goins *et al.*, 1997). Many studies carried out to evaluate the effect of LEDs on growth and development of plants, however, most studies focused on growth, photosynthesis, metabolism and gene expression (Neff *et al.*, 2000, Yu and Ong, 2003, Zhang *et al.*, 2003 and Wang *et al.*, 2009), while less dialed about how LEDs affects the survival rate and quality of grafted seedlings during the healing and acclimatization processes. Regarding irradiation by different LEDs during healing and acclimatization period. Therefore, the main aim of this work is to study the influence of different LED light colors and flux on growth and grafted-take of the grafted cucumber seedlings.

Materials and methods

The experiment was carried out at SEKEM Company, El-Sharkia Governorate, Egypt during 2019 season to study the influence of white, red, blue and green LEDs on growth and grafting efficiency of the grafted cucumber seedlings.

Cucumber and rootstock seeds

Cucumber (*Cucumis sativus* L.) 'Barracuda F1' was used as non-grafted control. This cultivar is widely grown in commercial production in Egypt. The hybrids 'Nun 6001', 'Strongtosa' and 'Tetsukabuto' (*Cucurbita maxima* × *C. moschata*), released by Nunhems Zaden (The Netherlands), Syengenta Seeds (The Netherlands) and Takii (Japan), respectively, were used as rootstocks. 'Nun 6001 F1' is one of the most popular rootstocks commercially available for cucumber grafting worldwide (Miguel *et al.*, 2004; King *et al.*, 2010).

Location description

Fig. 1 illustrates the grafting location, which consists of germination room, acclimatization room, trolleys, seedling trays, air conditioners, air blowers and grafted plants traysroniculture refrigerators,



Fig. 1. A plan view of grafting location.

Germination room

After sowing, the trays were immediately transferred to the germination room (3 days). The germination room is made of concrete with dimensions of 12.0X11.4 m. The room was provided with trolleys to carry the seedling trays, two air conditioners (Range temperature 15 – 40 °C– Power 5 hp 380V) for temperature control and mist system to increase relative humidity. The trolley was made of galvanized steel metal of 3 mm thickness with dimensions 1.5X1.0X2.0 m. The seedling tray holds 209 plants (11 rows X 19 cells) each cell 2.5X2.5X7.4 cm.

Acclimatization room

It is used to adapt the rootstock with scion, with dimensions of 19.5X14 m. The room was provided with 35 trolleys to carry the grafted plants trays, six air conditioners for controlling temperature and four air blowers (Model C.C.P. Parma – Flow Rate 1350 m^3 h^{-1} – RPM 2800 – Power 2.5 hp 380V 50Hz, Italy) for mixing air. Each trolley is provided with five shelves (the first is provided with white, the second with red, the third with blue, the fourth with red + blue, and the fifth with green LEDs). The grafted plants tray holds 150 plants (10 rows X 15 cells) each cell with dimensions of 4.0X4.0X6.2cm. White peat+perlite (70:30) was used as soilless media. The amount of chemicals in chemigation system was as described by Hoagland and Arnon (1950).

Treatments

The treatments include: five LED colors (white, red, blue, red + blue and green) with three photosynthetic photon fluxes (PPF) (25, 50 and 75 µmol m^{-2} s^{-1}). Air temperature, relative humidity and photoperiod were maintained at 26 °C, 90% and 14/10 h (light/dark), respectively (will be mentioned as T). The experimental design was a split plot with three replications.

Measurements

Temperature and relative humidity were monitored by using a HOBO Data Logger (Model HOBO U12 Temp/RH/Light – Range -20 to 70°C and 5 to 95% RH,) every hour. Ten cucumber seedlings representing each replicate aged 8 days after grafting were taken as recommended by Jang *et al.* (2011) to determine shoot length, leaves number, leaf area, chlorophyll content (SPAD) and shoot and root dry weight. Shoot length was measured by using a digital caliper (Model TESA 1p65- Range 0-150 mm ± 0.01 mm,). A planimeter (Model Placom KP -90 N- Range 0-10 m2 ± 0.2 %,) was used to measure the leaf area. Chlorophyll content was measured by using a chlorophyll meter (Model Minolta SPAD-502 - Accuracy ± SPAD unit,). Fresh shoots and roots of seedlings were weighed and dried in oven at 65 °C for 48 h then weighed. Also graft-take (efficiency of grafting) was calculated as follows (equation 1):

$$Grafting \text{ efficiency} = \frac{Number \text{ of surviving seedlings}}{\text{Number of grafted seedlings}} \times 100 \quad (1)$$

Statistical analysis

The data obtained were statistically analyzed according to Snedecor and Cochran (1980) and the treatments compared using Least Significant Differences (LSD) test at 95% confidence level (Gomez, 1984).

Results and discussion

Shoot length

Fig. 2 shows the effect of (T) on the shoot length of the grafted cucumber seedlings. The results indicated that the shoot length decreases with increasing the photosynthetic photon flux. As photosynthetic photon flux increased from 25 to 75 µmol m^{-2} s^{-1}, the shoot length non-significantly decreased from 8.60 to 8.45, 8.27 to 7.97, 8.98 to 8.67, 8.21 to 8.05 and 9.66 to 9.27 cm for white, red, blue, red + blue and green light colors, respectively. These results are in line with those obtained by Jang *et al.* (2011) who found the shoot length of grafted cucumber seedlings decreased from 9.65 to 7.45 cm when the photosynthetic photon flux increased from 0 to 237 µmol m^{-2} s^{-1}.

Regarding of the light colors effect, the highest value of shoot length (9.66 cm) was found for the seedlings grown under the green light, while the lowest value (7.97 cm) was obtained for the seedlings grown under the red light. This trend is agreed with those obtained by Jang *et al.* (2013) who found the lowest value of shoot length of the grafted cucumber seedlings under red light, and Kim *et al.* (2004) mentioned that the green light enhances the growth of lettuce

plants. The statistical analysis showed that, the differences between the obtained data of shoot length due to the effect of light colors (A) was significant, while the effect of photosynthetic photon flux (B) was non-significant, moreover the interaction between AB was significant.

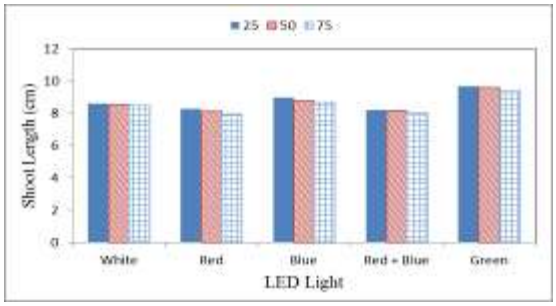


Fig. 2. The effect of (T) on the shoot length of the grafted cucumber seedlings

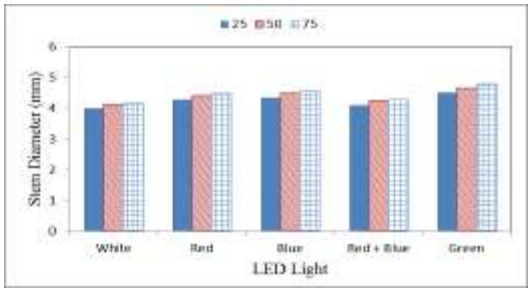


Fig. 3. The effect of (T) on the stem diameter of the grafted cucumber seedlings.

Stem diameter

Fig. 3 shows the effect of (T) on the stem diameter of the grafted cucumber seedlings. The results indicated that, the stem diameter increased with increasing the photosynthetic photon flux. As the photosynthetic photon flux increased from 25 to 75 µmol m^{-2} s^{-1}, the stem diameter non-significantly increased from 4.007 to 4.163, 4.282 to 4.485, 4.339 to 4.562, 4.111 to 4.294 and 4.506 to 4.785 mm for white, red, blue, red + blue and green light colors, respectively. These results agreed with those obtained by Nobuoka *et al.* (2005) and Jang *et al.* (2011) in which the stem diameter of grafted cucumber seedlings was enhanced by light photosynthetic photon flux during acclimatization. Regarding of the effect light colors, the highest value of stem diameter of the grafted cucumber seedlings (4.785 mm) was found under green light, while the lowest value (4.007 mm) was obtained under white light. These results agreed with those obtained by Kim *et al.* (2004) and Pardo *et al.* (2014). The statistical analysis showed that the differences between the stem diameter of the grafted cucumber seedlings due to the effect of light colors (A), photosynthetic photon flux (B) the interaction between AB were non-significant. .

Number of leaves

Fig. 4 shows the effect of (T) on the leaves number of the grafted cucumber seedlings. The results indicated that, the leaves numbers were 1.77, 1.84, 2.01, 1.93 and 2.19 for white, red, blue, red + blue and green light, respectively at 25 µmol m^{-2} s^{-1}. While, they were 1.89, 1.95, 2.06, 2.02 and 2.27 for white, red, blue, red + blue and green LED light, respectively at 50 µmol m^{-2} s^{-1} and they were 1.78, 1.88, 1.99, 1.97 and 2.21 for white, red, blue, red + blue and green light, respectively at 75 µmol m^{-2} s^{-1}.

The results also indicated that, the grafting at 50 µmol m^{-2} s^{-1} photosynthetic photon flux gave the best results in terms of leaves number (2.27 leaf/plant average). While, the lowest value of the leaves number (1.77 leaf/plant average) was obtained at 25 µmol m^{-2} s^{-1} .

The statistical analysis showed that, the differences between the leaves number of the grafted cucumber seedlings due to the effect of light colors (A), photosynthetic photon flux (B) and the interaction between AB were non-significant.

Leaf area

Fig. 5 shows the effect of (T) on the leaf area of the grafted cucumber seedlings. The results indicated that, the leaf area increased with increasing the photosynthetic photon flux from 25 to 75 µmol m^{-2} s^{-1}.

The leaf area significantly increased from 52.65 to 58.34, 46.36 to 49.68, 50.09 to 53.00, 52.22 to 56.04 and 48.65 to 52.71 cm^2 for white, red, blue, red + blue and green light, respectively. Plants grown under high photosynthetic photon flux (PPF) condition had higher photosynthetic capacity, greater leaf thickness and more chloroplasts in mesophyll cells.

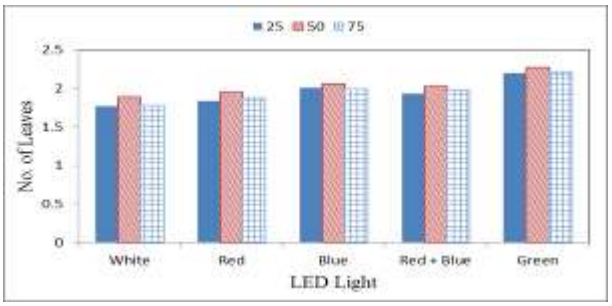


Fig. 4. The effect of (T) on the number of leaves of the grafted cucumber seedlings.

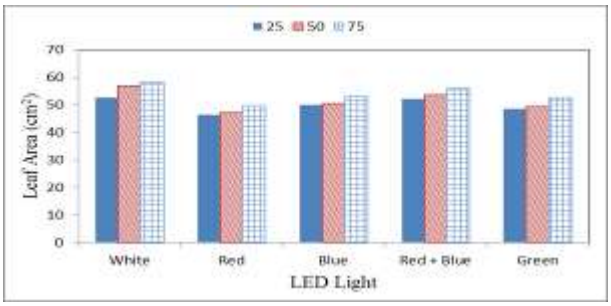


Fig. 5. The effect of (T) on the leaf area of the grafted cucumber seedlings.

Increasing the photosynthetic photon flux (PPF) during acclimatization increased the amount and distribution of chloroplasts in scion cotyledon, and they covered most of cell wall with little open space left, compared with that of dark condition. These results agreed with those obtained by Oguchi *et al.* (2003) and Jang *et al.* (2011). The highest value of leaf area (58.34 cm^2) was found under white light, while the lowest value (46.36 cm^2) was obtained under red light. This trend of these results agreed with those obtained by Jang *et al.* (2013). The statistical analysis showed that, the differences between the leaf area of the grafted cucumber seedlings due to the effect of light colors (A), photosynthetic photon flux (B) and the interaction between AB were significant.

Chlorophyll content

Fig. 6 shows the effect of (T) on the chlorophyll content (SPAD) of the grafted cucumber seedlings. The results indicated that the chlorophyll content increased with increasing the photosynthetic photon flux, where, the photosynthetic photon flux increased from 25 to 75 µmol m^{-2} s^{-1}, the chlorophyll content significantly increased from 24.33 to 27.21, 24.91 to 26.48, 22.80 to 24.45, 28.32 to 29.82 and 29.08 to 32.67 for white, red, blue, red + blue and green light, respectively. These results agreed with those obtained by Li and Kubota (2009).

The highest value of chlorophyll content (32.67) was found under green light, while the lowest value (22.80) was obtained under blue light.

The statistical analysis showed that, the differences between the chlorophyll content of the grafted cucumber seedlings due to the effect of light colors (A), photosynthetic photon flux (B) and the interaction between AB were significant.

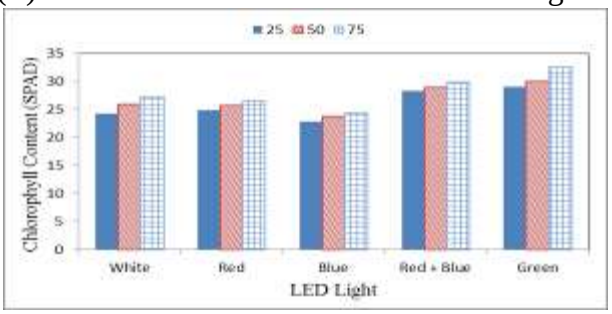


Fig. 6. The effect of (T) on the chlorophyll content of the grafted cucumber seedlings.

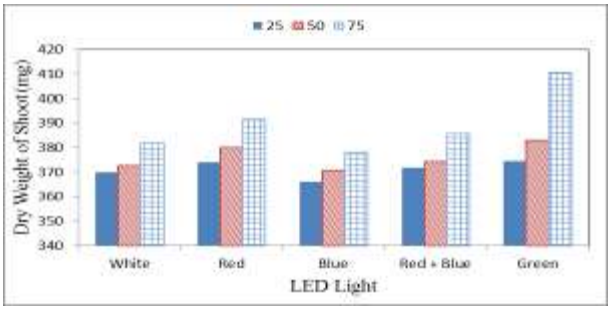


Fig. 7. The effect of (T) on the shoot dry weight of the grafted cucumber seedlings.

Shoot dry Weight

Fig. 7 shows the effect of (T) on the shoot dry weight of the grafted cucumber seedlings. The results indicated that, the shoot dry weight increases from 369.98 to 381.77, 374.06 to 391.72, 366.19 to 378.00, 371.88 to 385.66 and 374.53 to 410.39 mg with increasing the photosynthetic photon flux from 25 to 75 μmol m^{-2} s^{-1} for white, red, blue, red + blue and green light, respectively. The increase of photosynthetic photon flux during acclimatization led to the improvement of growth and quality of grafted cucumber seedlings. These results agreed with those obtained by Kim *et al.* (2001) and Jang *et al.* (2011). The highest value of shoot dry weight (410.39 mg) was found under green light, while the lowest value (366.19 mg) was obtained under blue light. These results agreed with those obtained by Jang *et al.* (2013). The statistical analysis showed that, the differences between the shoot dry weight of the grafted cucumber seedlings due to the effect of light colors (A), photosynthetic photon flux (B) and the interaction between AB were significant.

Grafting efficiency (grafted-take)

Fig. 8 shows the effect of (T) on the efficiency of grafting (grafted-take) of the cucumber seedlings. The results indicated that, the grafting efficiency decreases from 89.52 to 83.99, 78.23 to 72.83, 77.99 to 71.36, 83.62 to 80.01 and 76.08 to 69.78 % with increasing the photosynthetic photon flux from 25 to 75 μmol m^{-2} s^{-1} for white, red, blue, red + blue and green light, respectively. These results agreed with those obtained by Kim and Kim (2014).

The highest value of grafting efficiency (89.52 %) was found under white light, while the lowest value (69.78 %) was obtained under green light. These results agreed with those obtained by Kim and Kim (2014). The statistical analysis showed that, the differences between the grafted-take of the grafted cucumber seedlings due to the effect of light colors (A) was significant, photosynthetic photon flux (B) was non-significant and the interaction between AB was significant.

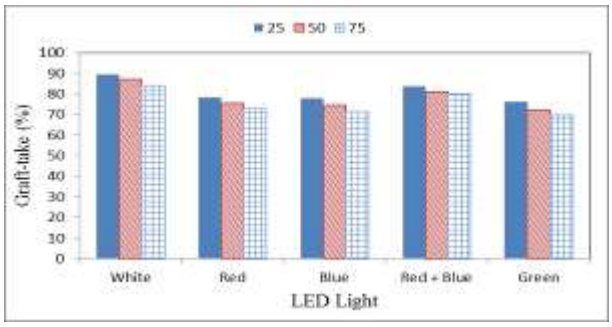


Fig. 8. The effect of (T) on the grafted-take of the grafted cucumber seedlings.

Simple linear regression analysis was carried out to estimate the equation expresses the relation between the grafted-take (dependant variable) and both of photosynthetic photon flux (PPF) (independent variable) at different light colors as following:

$$GT = aPPF + b \quad (2)$$

Where:

GT = the grafted-take of grafted cucumber seedlings, %

PPF = the photosynthetic photon flux, μmol m^{-2} s^{-1}

The constants of these equations and coefficients of determination are listed in Table 4.

Table 4 The constants a, b and coefficient of determination for grafted-take at the different photosynthetic photon flux (PPF) at different light colors.

Light color	Constants		R^2
	a	b	
White	-2.765	92.423	0.993
Red	-2.700	80.870	0.994
Blue	-3.315	81.217	0.998
Red + Blue	-1.805	85.140	0.930
Green	-3.150	78.993	0.983

Conclusion

The obtained results indicated that the shoot length decreases with increasing photosynthetic photon flux, the highest value of shoot length was 9.66 cm was obtained at 25 µmol m^{-2} s^{-1} under green light. The stem diameter and leaf area increases with the increasing of photosynthetic photon flux, the stem diameter and leaf area ranged from 4.007 to 4.785 mm and 46.36 to 58.34 cm^2, respectively. The highest value of the leaves number of the grafted cucumber seedlings (average 2.27 leaf) was obtained at 50 µmol m^{-2} s^{-1} under green LED light. The chlorophyll content ranged from 22.80 to 32.67 for all treatment. The shoot dry weight of the grafted cucumber seedlings ranged from 366.19 to 410.39 mg for all treatment. The grafted-take decreases with increasing photosynthetic photon flux, the highest value of grafted-take was 89.52 % was found at 25 µmol m^{-2} s^{-1} under white LED light

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PHENOLOGICAL CHARACTERISTICS OF SWEET CHERRIES ON TWO DIFFERENT TYPES OF EUTRIC CAMBISOL IN HERZEGOVINA REGION (BOSNIA AND HERZEGOVINA)

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Abstract

The aim of this study was to determine the influence of different soil types of eutric cambisol on flowering and ripening of sweet cherry (Prunus avium) cultivars (Burlat, Skeena and Sweet heart). The research was conducted on the micro-locality Vrapčići, north of Mostar city (Bosnia and Herzegovina) in 2018 and 2019. The influence of the year on the beginning and duration of the flowering phenophase was evident, and the earlier beginning and shorter duration of flowering in all cultivars was recorded in 2019. Soil type had a statistically significant impact on the beginning of flowering, so the earliest beginning of flowering was recorded on soil variety I, in the cultivar Burlat in 2019, and the latest in the cultivar Skeena on the type II soil in 2018. The cultivars Burlat and Sweet heart had the shortest flowering in 2019, and in 2018 Burlat flowered the longest on soil type I and Sweet heart on soil type II. Ripening took place at the same time on both soil types. The earliest beginning of ripening was in the cultivar Burlat in 2019, and the latest in the Sweet heart cultivar in 2018. The shortest ripening duration was recorded in the Sweet heart cultivar, while the longest ripening duration in the Burlat cultivar in both observed years. It can be concluded that the soil types I and II significantly influenced the beginning of flowering of all three cultivars of sweet cherry (Burlat, Skeena and Sweet heart) in both study years, while the type of soil has had no impact on the stages of ripening phenophase.

Keywords: *soil type, sweet cherry, flowering, ripening.*

Introduction

One of the oldest economically-exploited fruit species in Bosnia and Herzegovina is the sweet cherry (*Prunus avium* L.). Compared to other fruit species, it is characterized by earlier ripening of the fruit and a pleasant taste, nutritional value and low caloric content. According to the number of trees in relation to other fruit species, sweet cherry ranks fourth in Herzegovina. Twenty years ago in Herzegovina, a period of intensive cultivation of sweet cherries began in a dense formation. Considering that the sweet cherry is a foreign-fertile species and that its flowering depends on the agro-ecological conditions of the certain area, monitoring the dynamics of flowering and ripening is of great importance for producers. This topic has been the subject of research by several researchers (Horvat et al., 2008; Aliman, 2008; Usenik and Fajt, 2008; Aliman and Drkenda, 2009; Milatović et al., 2013; Aliman et al., 2013).

The new method of cultivation requires a different approach in relation to the choice of terrain position, soil, substrate, variety, irrigation, protection, fertilization and the use of mechanization, formation of the desired cultivation form, organization of harvesting, and all for the benefit of the high yields. A lot of resources are invested in production, which requires continuous work and constant monitoring of plantations.

The aim of this study is to show impact of two types of Eutric Cambisol soil, on the dynamics of flowering and ripening of the three sweet cherry cultivars (Burlat, Skeena and Sweet heart) that have a different ripening time and are grafted on a medium lush rootstock Colt.

Materials and Methods

The research was conducted during 2018 and 2019 at the location "Vrapčići", north of Mostar (Bosnia and Herzegovina). The experimental plot has an altitude of about 60 m and is characterized by flat terrain. The soil type is Eutric Cambisol, i.e. brown carbonate skeletoid shallow and medium deep valley soil on gravels and serpentines. The plant was established in 2009 and is in the period of full fruiting. The research was conducted on three sorts of sweet cherries (Burlat, Skeena and Sweet heart) grafted on a Colt rootstock whose planting distance in the plantation is 4.5 x 2.5 m. The breeding form is a slender spindle. According to the classification of breeding systems on the degree of intensity, this plant is currently classified as intensive.

The research included the flow of flowering phenophases by a visual method (Štampar, 1956) and ripening (beginning and end) of three cultivars grafted on Colt rootstock, that were grown on two types of Eutric Cambisol soil. The beginning of flowering was recorded when the first flowers were open (10%), full flowering (most open flowers - 90%) and the end of flowering (when 90% of the corolla leaves fell). Based on the obtained data for each investigated cultivar, year and soil type, a flowering phenogram was made.

Results and discussion

Characteristics of the investigated types of Eutric Cambisol

The Eutric Cambisol soil is found on the observed micro-locality, with its two types established based on clay displacement.

The profile structure of the Eutric Cambisol is Ah-Bv-IC. The depth of the profile Ah-Bv, which is part of the soil profile, is 50-100 cm. The pedophysical characteristics of these soils are good to very good. They have good chemical characteristics, slightly acidic to neutral soil reaction. There is an evident difference in the investigated locality, so the Eutric Cambisol is divided into two soil types (type 1 and 2). The division into is based on clay displacement. Type 1 - Brown carbonate skeletal medium deep soil on gravels and serpentines. It is formed by eroding in layers from the surrounding hills. As these are younger soils, a shallower or deeper (A), and Ap-M horizon is formed. It is characterized by the presence of texturally lighter material in the layers. It contains about 50% of the skeleton that grows with depth. The skeleton is present in almost the entire depth of the soil profile and the soil is very permeable. According to the texture composition, they are sandy-clay loams. Type 2 - Brown carbonate skeletal shallow soils on gravels naturally bind to alluvial carbonate soils so that they have the same origin. These are deep, more or less skeletal soils of very good physical properties, especially in terms of absolute air capacity. The mechanical composition is quite uneven and they represent clays and loams with a smaller percentage of sand. The parent substrate has a high carbonate content (about 20%), so the current soil reaction is alkaline. The humus content in the surface horizon is mediocre. Table 1 shows the chemical composition of two Eutric Cambisol soil types, sampled from two depths, at the experimental site in Vrapčići.

Table 1: Chemical properties of soil at the experimental locality Vrapčići

Soil type	Eutric Cambisol			
Depth (cm)	Type 1		Type 2	
	0-30	30-60	0-30	30-60
pH in H_2O	7,48	7,09	8,20	8,24
pH in KCl	6,23	5,52	7,09	7,67
Total lime (%)	1,00	1,00	4,00	15,00
Org. substance (%)	3,00	2,00	2,00	2,00
Total N (g/kg)	0,44	0,34	0,38	0,32

Table 2 shows the textural composition of both soil types on the experimental plot in Vrapčići.

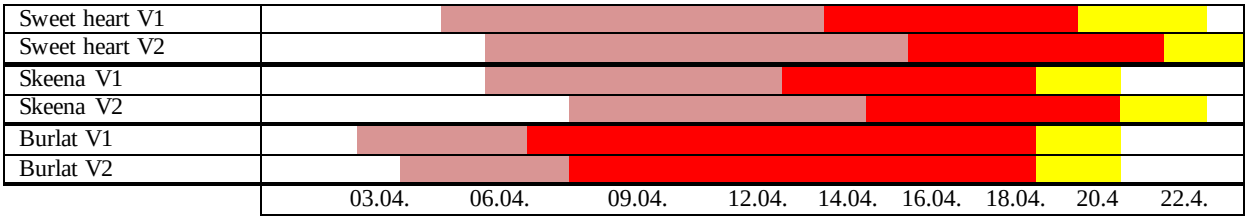
Table 2: Textural composition of the soil at the experimental site in Vrapčići

Soil	Depth (cm)	Textural composition of the soil %			Texture mark (USDA)
		sand	clay	dust	
Type 1	0-30	49	38	13	loam
	30-60	47	40	13	Ioam
Type 2	0-30	59	32	9	loam
	30-60	61	33	6	sandy-loam

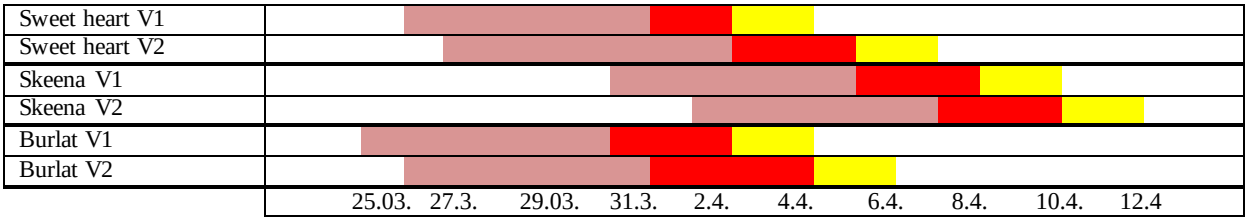
Based on the data presented in Table 1, it is evident that type 2 of soil has a lower pH value and lime content at both depths compared to type 2. The basic character of type 2 of soil can be explained by its anthropogenization over a long period of time involving fertilizer application, which affects the pH value of the soil. Analysis of the texture composition (Table 2) of both soil depths shows that type 2 of soil has a lower clay content compared to type 1.

Phenological characteristics of the sweet cherry cultivars

The phenological tests included monitoring the course of flowering and ripening phenophases: the beginning of flowering, full flowering and the end of flowering, duration of flowering on two observed soil types of the Eutric Cambisol, and the beginning, end and the duration of ripening. Flowering phenograms of the examined sweet cherry cultivars are presented in Graphs 1 and 2.



Graph 1: Phenogram of flowering of examined sweet cherry cultivars in 2018 on two soil types

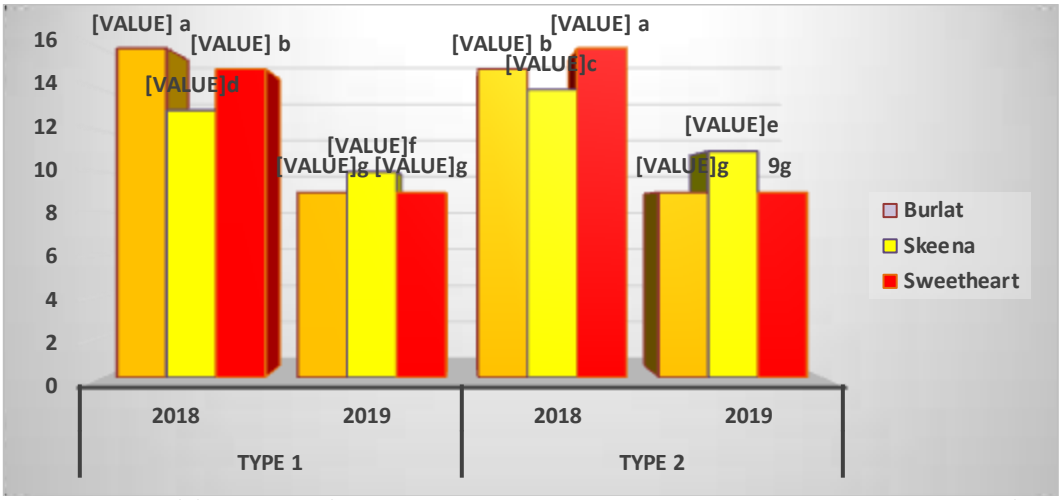


Beginning of flowering; Full bloom; End of flowering; V1-soil variety 1; V2- soil variety 2

Graph 2: Phenogram of flowering of tested sweet cherry cultivars in 2019 on two soil types

Based on the data on the time of flowering, it can be stated that the earliest beginning of flowering in both years was recorded in the cultivar Burlat, and the latest in the cultivar

Skeena on both soil types. According to Gadže et al. (2011), the onset of sweet cherry blossoming is conditioned by the genetic predisposition and weather conditions, especially the air temperatures that precede flowering, and during the dormant winter period.
Considering that March 2019 was very dry with average daily temperatures above average, this was reflected in the earlier start, full flowering and end of flowering in 2019 compared to 2018. Type 1 of soil showed a slightly earlier course of flowering phenophase (beginning, full and end) compared to type 2 of soil in all cultivars except for the end of flowering in Burlat in 2018, which was recorded on the same day for both soil types. Considering that flowering overlap is extremely important for successful fertilization, all species partially or completely overlapped, except for the beginning of flowering of Burlat and Skeena sorts on type 2 in 2018 and on type 1 in 2019.
The obtained data on flowering time of Burlat cultivar are consistent with the results obtained by Radunić et al., (2008) in the area of Kaštela, as are the flowering results for Skeena variety with the results obtained by Gadža et al., (2011) in Zagreb in Croatia, as well as the results of Milatović et al. (2013) in the area of Mačva in Serbia. Flowering time for the Sweet heart variety coincided with the results of Sarisu et al. (2016) in Turkey.



Graph 3. Duration of flowering of Burlat, Skeena and Sweet heart sorts during two years of research on two types of Eutric Cambisol

It is evident in the chart 3 that the duration of flowering for all observed cultivars was directly dependent on the climatic conditions of the observed year. In 2018, a significantly longer flowering period was recorded for all cultivars compared to 2019 on both observed soil types. Differences in terms of flowering duration between cultivars and years are statistically significant except for cultivars Sweat heart and Burlat in 2019 on both soil types. On type 1 of soil, a slightly shorter duration of the flowering phenophase in 2018 was recorded for cultivars Skeena and Sweet heart compared to type 2 of soil, in contrast to cultivar Burlat, which in 2018 showed a longer flowering period on type 1 of soil. The obtained data on the duration of flowering of the Skeena cultivar are in line with the results of Gadže et al. (2011) and Milatović et al. (2013).

Table 3. Phenophase of ripening of Burlat, Skeena and Sweet heart cultivars (2018-2019)

soil Eutric Cambisol	Year	Beginning			The end			Duration of ripening		
		Burlat	Skeena	Sweetheart	Burlat	Skeena	Sweetheart	Burlat	Skeena	Sweetheart
type 1	2018	6-May	1-Jun	17-Jun	17-May	7-Jun	20-Jun	13	6	3
	2019	2-May	3-Jun	14-Jun	13-May	10-Jun	19-Jun	11	7	5
type 2	2018	6-May	1-Jun	17-Jun	17-May	7-Jun	20-Jun	13	6	3
	2019	2-May	3-Jun	14-Jun	13-May	10-Jun	19-Jun	11	7	5

Reviewing the data from Table 3, it can be concluded that the beginning, end and duration of ripening in the observed period (2018-2019) flowed simultaneously on both soil types in the all cultivars. The earliest beginning of ripening in both years was the cultivar Burlat as the earliest of the examined cultivars (May 6, 2018 and May 2, 2019), then the cultivar Skeena as a cultivar of medium early ripening (June 1, 2018 and June 3, 2019), and the latest beginning of ripening was in the cultivar Sweet heart (June 17, 2018 and June 14, 2019). The harvest time for the Skeena cultivar is slightly earlier compared to the results of Gadže et al. (2011) and Milatović et al. (2013) as well as Sweet heart cultivar in relation to the results of Sarisu et al. (2016) in Turkey, which can be explained by the sub-Mediterranean climate in the area of Mostar.

Conclusion

Based on the obtained results, it can be concluded that the observed phenophases were greatly influenced by the year of observation, as well as the genetic predisposition of the species. The influence of soil type was recorded in relation to the time of flowering, but not to the time of ripening. For more precise conclusions and recommendations on the impact of soil types, this research should continue in the coming period.

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THE EFFECT OF WATER EXTRACT OF ALOE VERA (L.) BURM. F. ON GERMINATION AND GROWTH OF SCARLET SAGE

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Abstract

The aim of research was to determine effect of water extracts of plant species *Aloe vera* (L.) Burm.f. on the seed germination, germination energy and growth of scarlet sage seedlings- *Salvia splendens* L. The seed that has better germination energy also has better vigor, so it is more resistant to stressful conditions during germination. *Aloe vera* is an important medicinal plant from the Liliaceae family. It is a succulent herb which grows world wide. Its large leaves contains liquid of yellow latex and clear gel, which is rich in essential amino acids, mono- and polysaccharides, lignin, macronutrients, micronutrients, vitamins, gibberellins and salicylic acid. Aloe leaf extract has been used to improve the vegetative growth of many horticulture plants. Aloe extract contains plant hormones such as gibberellin, and can be used as a source of hormone instead of synthetic growth regulators. The experiment was conducted in laboratory condition at the Faculty of Agriculture, University of Banja Luka and consisted of control (distilled water) and treatment with water extract of Aloe. Water extracts were prepared from fresh leaves and experiments were conducted in Petri dishes at the 5, 10, 15, 20%. Temperature in the laboratory was constant at 20°C and light regime was set to 16h day/8h night. Water extracts of Aloe at a concentration of 20% had positive effect on the seed germination and germination energy, while concentration of 10% had positive effect on shoot and root length, and fresh and dry mass of scarlet sage seedlings.

Key words: *scarlet sage, Aloe vera (L.) Burm. f., water extracts.*

Introduction

The first record of human use of *Aloe vera* is on clay tablets during the Mesopotamia civilization in which it is described as a laxative. Use of aloe in ancient times is also documented in Egypt, Greece, and China. *Aloe vera* was cultivated on the islands of Barbados and Curacao in the Caribbean by Spain and the Netherlands and was sold in various parts of Europe during the 17th century (Park and Jo, 2006). *Aloe vera* originated in the warm, dry climates of Africa, the plant is readily adaptable and grows worldwide (Steenkamp and Stewart, 2007). Although its shape looks like a cactus, it belongs to the lily family. Genus *Aloe* includes more than 400 species, among which they are the most famous *Aloe vera* (L.) Burm. f., *Aloe arborescens* Miller. and *Aloe vera* var. chinensis (Haw.) A. Berger (Barišić *et al.*, 2014).

Aloes are perennial succulents and they can adapt to habitats with low or erratic water availability. They are characterized by possibility to store large volumes of water in their tissue. Aloe plants have green fleshy leaves covered by a thick cuticle or rind, under which is a thin vascular layer covering an inner clear pulp. The leaves are 30-50 cm in length and 10 cm in width at the base, pea-green in colour and with bright yellow tubular flowers 25-35 cm in length (Boudreau *et al.*, 2013). The main feature of the *Aloe vera* plant is its high water

content, ranging from 99% to 99.5%, while the remaining 0.5–1.0% solid material is reported to contain over 200 different potentially active compounds, including vitamins, minerals, enzymes, simple and complex polysaccharides, phenolic compounds, and organic acids (Boudreau *et al.*, 2013; Rodríguez *et al.*, 2010).

Three types of *Aloe vera* extracts can be distinguished: gel extract, whole leaf extract, and decolorized whole leaf extract (Boudreau *et al.*, 2013). This plant has many pharmacological properties including antioxidant, antibacterial, antifungal and immunomodulatory effects (Hosseinimehr *et al.*, 2010). Use of *Aloe vera* gel extracts in health foods and beverages, and moisturizing cosmetics, began during the 1970s, starting in the USA and parts of Europe (Park and Jo, 2006). The *Aloe vera* plant has been used in folk medicine for more than 2000 years, and it remains an important component of traditional medicine in many contemporary cultures, such as China, India, the Caribbean, and Japan (Grindlay and Reynolds, 1986). *Aloe vera* extracts may be used in beverages as bitter flavouring agent (O'Neil *et al.*, 2006), or in some dietary supplements and food products (Steenkamp and Stewart, 2007). The gel is used in the cosmetics industry as a hydrating ingredient in liquids, creams, sun lotions, lip balms, hair tonic, shampoo, and skin-moistening gel (Newton, 2004).

More than 75 bioactive components have been identified in the plant and Alipoor *et al.* (2012) believe that several chemical components are responsible for allelopathy of *Aloe vera*. Vukadinović *et al.* (2014) report that allelochemicals as secondary metabolites with multiple functions protect plants from various biotic and abiotic environmental stresses. The application of negative allelopathic influence is an alternative to the use of chemical agents in pest control in integrated and ecological production systems (Chon *et al.*, 2005). On the other hand, allelochemicals with a positive effect can be used as biostimulators and biofertilizers to improve crop growth, development and yield (Lin *et al.*, 2004; Popa *et al.*, 2008; Bhadha *et al.*, 2014).

The aim of research was to determine effect of water extracts of plant species *Aloe vera* (L.) Burm.f. on the seed germination, germination energy and growth of scarlet sage seedlings-*Salvia splendens* L.

Material and Methods

Investigation was conducted in May 2020 in laboratory conditions at the growing chamber in the Faculty of Agriculture, University of Banja Luka (Entity of Republic of Srpska, Bosnia and Herzegovina). Flower seed of *Salvia splendens* L. (manufacturer PanAmerican Seeds) was used. The seeds were disinfected with 1% NaOCl for 20 minutes before the experiment and washed three times with distilled water. The water extracts of plant species *Aloe vera* (L.) Burm.f. used in the experiment were prepared according to the method of Hanafy *et al.* (2012) from *Aloe vera* leaves. *Aloe vera* fleshy leaves was obtained, the two side margins were removed, the remainder was cut to pieces and blended in a blender. The mixture was placed in a sterile strainer and squeezed powerfully. The juice was obtained in a glass beaker and various water extracts of Aloe were obtained by diluting the juice with distilled water.

Experiment was set up in Petri dishes sterilized with 96% ethanol and lined with filter paper which is sprayed with 3 ml of distilled water (control group) or with 3 ml of 5, 10, 15 and 20% water extract of *Aloe vera* (treatment). Twenty-five seeds were counted and placed in each Petri dishes on the wet paper. Experiment was set-up in four repetitions for each treatment. There were a total of 500 seeds in the experiment. Prepared Petri dishes with seeds were placed in the growth chamber under an artificial white light for 16h a day and 8h per night. Temperature during the research was constant (20±1°C). Petri dishes were observed daily and additional distilled water or Aloe water extract was added if needed. Seeds were kept under these conditions for 14 days. After 7 days germination energy and after 14 days

germination of the seeds were tested. Both values are expressed as percentage. The number of developed cotyledons were recorded during the research. At the end of the experiment shoot and root length (cm) was measured using graph paper, and fresh and dry mass (g) of scarlet sage seedlings was obtained with an electronic scale. The data obtained was statistically analysed (LSD, F-test, t-test) using standard computer programs and VVSTAT (Vukadinović, 1994).

Results and Discussion

Statistically analyzed obtained results of seed germination, germination energy, number of cotyledons, shoot and root length, and fresh and dry mass of Salvia splendens L. seedlings under influence of Aloe water extracts were given in table 1.

Table 1: Influence of water extracts of plant species Aloe vera (L.) Burm.f. on the seed germination, germination energy and number of cotyledons of scarlet sage seedlings - Salvia splendens L. (means marked with different letters (^{a,b,c,d}) significantly differ at p= 0.05)

Treatment variant	germination energy %	germination %	No. of cotyledons
Control K	72^{b}	77^{b}	14.31^{b}
Treatment T_1	66^{c}	73^{c}	13.31^{c}
Treatment T_2	64^{c}	67^{d}	13.13^{c}
Treatment T_3	60^{d}	65^{d}	12.19^{d}
Treatment T_4	75^{a}	79^{a}	15.31^{a}
Average	67.40	72.20	13.65
Analysis of variance - F	5.13^{**}	5.12^{**}	5.13^{**}
LSD	germination energy %	germination %	No. of cotyledons
0.05	8.07	8.12	1.59
0.01	11.16	11.23	2.20

Data shown in Table 1. indicates very significant difference (p=0.01) between the average values of germination energy, seed germination and number of developed cotyledons. Treatment T_4 - water extracts of Aloe at a concentration of 20% had the best result on all of three parameters. The average values germination energy of scarlet sage seeds in T_4 treatment were 75%, in control plants 72% while in other treatment average values germination energy were lower than in control group. The same ratio was in seeds germination, where the best result had T_4 with 79%, than control group with 77%, while with increasing concentration of other water extracts of Aloe the energy of seed germination decreased. The highest average number of developed cotyledons were 15.31 in T_4 treatment, in control group were 1.31, while the lowest average number of developed cotyledons were 12.91 in T_3 treatment with 15% of Aloe water extract (Table 1.).

Table 2: Influence of water extracts of plant species Aloe vera (L.) Burm.f. on the growth of scarlet sage seedlings - Salvia splendens L. (means marked with different letters (^{a,b,c,d}) significantly differ at p= 0.05)

Treatment variant	shoot lenght (cm)	root lenght (cm)	fresh mass (g)	dry mass (g)
Control K	0.63^{a}	0.55^{d}	0.50^{d}	0.04^{a}
Treatment T_1	0.62^{a}	0.59^{c}	0.53^{c}	0.04^{a}
Treatment T_2	0.63^{a}	1.96^{a}	0.79^{a}	0.05^{a}
Treatment T_3	0.58^{a}	0.62^{b}	0.56^{b}	0.04^{a}
Treatment T_4	0.58^{a}	0.43^{e}	0.48^{e}	0.04^{a}
Average	0.61	0.83	0.57	0.04
Analysis of variance - F	1.25	41.82**	6.80**	1.40
LSD	shoot lenght (cm)	root lenght (cm)	fresh mass (g)	dry mass (g)
0.05	ns	0.29	0.14	ns
0.01	ns	0.41	0.20	ns

In the Table 2. data shown influence of water extracts of Aloe on the growth of scarlet sage seedlings: shoot lenght (cm), root lenght (cm), fresh mass (g) and dry mass (g). Treatment T_2 - water extracts of Aloe at a concentration of 10% had the best result on all of four parameters. Statistical analyses of the results show very significant difference (p=0.01) between the average values of root lenght and fresh mass for the control plants and treatment with different percentage of Aloe water extract. The highest average value of root lenght was observed in T_2 treatment 1.96 cm, while the lowest average value of root lenght was in treatment T_4 0.43 cm (20% water extract of Aloe). The same ratio was in average value of fresh mass, where the highest average value of fresh mass was in T_2 treatment 0.79 g and the lowest in treatment T_4 0.48 g. The highest average values of shoot lenght (0.63 cm) and dry mass (0.05 g) were observed in treatment T_2 (10% water extract of Aloe) but this values were not sufficient to be statistically proven (Table 2.).

The positive influence of extracts from species of the genus Aloe on the growth and development of plant species has been noted by many authors, so according to El-Shayeb (2009) extracts of *Aloe vera* species in concentrations of 25, 50 and 75% increased height, number of branches, number of leaves, fresh and dry mass of leaves, and the fresh and dry mass of the stem common evening-primrose (*Oenothera biennis*). Hanafy et al. (2012) report a positive effect on growth parameters in flowering and ornamental species. *Aloe vera* leaf gel is a very excellent source of plant nutrients: calcium, iron, magnesium, potassium, phosphorous and zinc (Dagne *et al.*, 2000); amino acids: alanine, glycine, leucine and proline (Reynolds and Dweck, 1999); vitamins-B complex, C, β-carotene and α-tocopherol (Vinson *et al.*, 2005) and other organic compounds: triglicerides, triterpenoid, gibberillin, potassium sorbate and salicylic acid (Hamman, 2008). The importance of allelopathic influence of *Aloe vera* water extract as a natural weed control in plant protection and biostimulators in plant production has been confirmed by numerous studies. The results of the experiment showed that extracts of fresh leaves of *Aloe vera* have stimulating allelopathic influence of scarlet sage, which confirms the presence of allelochemicals. Leaf extracts of species Aloe can be useful as natural growth regulators (Lin *et al.*, 2004), and the allelopathic effect can be

attributed to the presence of allelochemicals, such as tannins, flavonoids and phenolic acids (Alipoor *et al.*, 2012).

Conclusions

Based on the obtained results we can conclude that the treatment with a certain percentage water extract of Aloe can enhance seed vigor and plant productivity of scarlet sage - *Salvia splendens* L.

Water extracts of Aloe at a concentration of 20% (T_4) had very statistically significant effect on the seed germination, germination energy and number of developed cotyledons. Concentration of 10% (T_2) had very statistically significant effect on root length and fresh mass of scarlet sage seedlings. Treatment T_2 did not show statistically significant effects on shoot lenght and dry mass, but the average values had slightly allelopathic influence than other treatments or control. We can conclude that the 20% water extract of Aloe is recomended in seed germination, but 10% water extract can be used in trasplantation phase, like biostimulants, to enhance shoot and root lenghts of scarlet sage - *Salvia splendens* L. The observed positive effects of the presence of allelochemicals may be the subject of further research in the form of a plant stimulator of crop growth and development.

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RESPONSE OF SOME WINTER WHEAT CULTIVARS TO NITROGEN TOPDRESSING AND SOWING DENSITY

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Abstract

The aim of study was to assess the effects of interaction between nitrogen fertilization and sowing densities on grain yield of modern wheat cultivars. A two-year study of the wheat response to increasing doses of nitrogen fertilization and different sowing densities was carried out under rainfed conditions in the experimental field of the Institute of Field and Vegetable Crops, Novi Sad, Serbia. The experiment consisted of four nitrogen fertilization treatments (F): 0, 50, 100 and 150 kg N ha^{-1}; four winter wheat cultivars (C): NS 40S, NS Azra, NS Ilina and NS Petrija, and four sowing densities (D): 300, 500, 700 and 900 viable seeds m^{-2}. Analysis of variance showed statistically significant effects of cultivars and sowing densities, as well as interactions F×C, C×D and F×C×D on total variability of grain yield. Contribution of cultivars to the total sum of squares was highest, showing different response to various N-fertilization and sowing density treatments. On average, grain yield varied between cultivars from 9.82 (NS Petrija) to 10.74 t ha^{-1} (NS 40S). The highest grain yield was achieved with cultivar NS 40S under conditions of 100 kg N ha^{-1} and 900 viable seeds m^{-2}. However, in most cases differences between 500, 700 and 900 viable seeds m^{-2} were not statistically significant.

Keywords: *Winter wheat, yield, nitrogen topdressing, sowing density*

Introduction

Among the most relevant management factors for improving wheat yields and closing the yield gap are adequate mineral nutrition and the use of the optimum seeding rate for an appropriate plant density (Jaenisch et al., 2019; Lollato et al., 2019). Nitrogen is a primary nutrient limiting the grain yield of winter wheat and represents a significant cost for the growers. For all agricultural systems, especially in areas of higher production there is a constant need for adequate amounts of nutrients, mostly supplied as fertilizers. Recently, agricultural practices have focused on maximizing yields by increasing N-fertilization (Jaćimović et al., 2013; Zhang et al., 2016). Moreover, nitrogen is the most limiting nutrient for wheat production that affects grain yield and biomass production, as well as establishment of high grain weight and grain number per unit area (Lawlor et al., 2001). A better understanding of relationship between wheat grain yield and plant density could improve plant density recommendations according to specific environmental and genetics characteristics. Below-optimum seeding rates may reduce resource use efficiency, yield and final profit, and above-optimum seeding rates increase cost of production and might potentially decrease yield by increasing disease pressure, insects, and lodging (Lloveras et al., 2004; Laghari et al., 2011). Optimum plant densities vary greatly between areas, climatic conditions, soil, sowing time, and varieties. Wheat yield components have a strong compensation capacity depending on the availability of resources. However, this compensatory mechanism could differ across wheat genotypes (Aćin et al., 2019).

Consequently, defining the agronomic optimum plant density is crucial for future improvements in wheat yield (Bastos et al., 2020). Therefore, the objectives of this study were to quantify the variation in grain yield of the new developed wheat cultivars across different N-fertilization levels and sowing densities in order to improve wheat production under agroecological conditions of Pannonian Plain.

Materials and methods

Field trials with four winter wheat cultivars, increasing nitrogen topdressing rates and sowing densities were carried out in two successive growing seasons (2016/17 and 2017/18), under rainfed semi-arid conditions of the southern Pannonian Plain in the Vojvodina Province. The main objective of this study was to analyze the effects of N-topdressing, cultivars and sowing densities, as well as their interaction on wheat grain yield (GY). The trials were setup at experimental fields of Institute of Field and Vegetable Crops, Novi Sad (45°19'49" N; 19°49'53" E; 80 m a.s.l.). Treatments were arranged in a split-split-plot design with three replications. Main plots were assigned to the nitrogen topdressing rates, sub-plots to cultivars and sub-sub-plots to sowing densities. N-fertilization treatments (F) included unfertilized control (0 N) and three fertilization levels with 50 (50 N), 100 (100 N) and 150 kg N ha^{-1} (150 N). The second factor included four winter wheat cultivars (C): NS 40S, NS Azra, NS Ilina and NS Petrija, released by the Institute of Field and Vegetable Crops, while the third factor consisted of four sowing densities (D): 300, 500, 700 and 900 viable seeds per square meter. Each plot consisted of 10 rows, with row spacing of 0.10 m and length of 5 m. The soil type at the experiment site was a calcareous chernozem. The contents of sand, silt and clay in the soil were recorded as 37, 38 and 25%, respectively. The reaction of soil solution was slightly alkaline (pH = 7.52), with moderate content of humus (3.42%) and available phosphorus (15.46 mg/100 g of soil) and with high content of available potassium (25.64 mg/100 g). The soil was medium provided by total nitrogen content (0.19% N). Weather data were collected from the meteorological station located near the experimental fields. Air temperature, level and distribution of precipitation in autumn enabled fast emergence and establishment of good plant population in both growing season. Winter of 2016/17 was characterized by low temperature (especially in January), while winter of 2017/18 were moderately cold. Winter weather conditions in both years provided a high percentage of overwintering (averaged nearly 90%). Temperature in spring growing period was similar in both years. Previous analysis of the effects of years on GY at different N-rate and sowing densities (without considering the effect of cultivars) where not significant (data not shown), so the results of the experiment are presented as an average of both years. Preceding crop in both growing seasons was soybean. According to the results of soil analysis a fertilizer combination (NPK 11:52:0) was applied before ploughing in both growing seasons, while N-topdressing rates were applied at the beginning of March, before the beginning of stem elongation. Wheat cultivars were sown on recommended sowing date (mid-October). Pests, weeds and diseases were prevented or controlled by applying the recommended pesticides. Fusarium and symptoms of other diseases were not observed or were insignificant. All of the experimental units were combine-harvested at maturity stage (early July) and grain moisture content was corrected to 130 g kg^{-1}. Analysis of variance of GY was performed using GenStat 12^{th} ed. (VSN International, Hemel Hempstead) while the treatments means were compared using Duncan's multiple range test.

Results and Discussion

According to ANOVA (Tab. 1), cultivars (C) and sowing densities (D), as well as interactions F×C, C×D and F×C×D showed statistically significant effects on the total GY variability,

indicating different cultivar responses to N-fertilization (F) and sowing density (D). Cultivars explained 28% of total GY variance, while contribution of F×C and C×D interactions was lower, but significant, showing different response of cultivars to analyzed treatments. Similar observations are reported by Mirosavljević et al. (2019) and Aćin et al. (2019), emphasizing the necessity of adapting management practices to each individual cultivar.

Table 1. The ANOVA of wheat grain yield and relative contribution to the total sum of squares (%) of the main factors: N-fertilization (F), cultivar (C), sowing density (D) and their interaction

Source of variation	d.f.	s.s.	s.s. (%)	m.s.	v.r.	F pr.
F	3	2.104	2.8	0.701	0.54	0.670
C	3	20.995	27.6	6.998	26.89**	<0.001
F × C	9	6.571	8.6	0.730	2.81*	0.021
D	3	5.821	7.7	1.940	13.03**	<0.001
F × D	9	1.574	2.1	0.175	1.17	0.320
C × D	9	2.998	4.0	0.333	2.24*	0.026
F × C × D	27	7.413	9.7	0.275	1.84*	0.016

d.f. - Degrees of freedom, s.s. - Sum of squares, m.s. - Mean square, v.r. - Variance ratio (F-test), F pr. - Probability value corresponding to a variance ratio; * significant at 0.05; ** significant at 0.01.

The average GY in the trial (grand mean) for all treatments was 10.22 t ha^{-1} (Tab. 2). N-topdressing rates significantly altered GY of cultivars, resulting in GY increase in comparison with the control treatment. On average, the highest GY was observed at treatments 100 N (10.39 t ha^{-1}) and 150 N (10.23 t ha^{-1}), while absence of N application resulted in the lowest GY (10.10 t ha^{-1}). However, a comparison based on the Duncan's test showed no statistically significant differences in GY between N-rates of 150, 50 and 0 kg N ha^{-1}.

Table 2. Response of wheat grain yield (t ha^{-1}) to nitrogen topdressing, cultivars and sowing densities

N-fertilization (F)	Cultivar (C)	Sowing density (D) 300	500	700	900	Average (F × C)	Average (F)
0 N	NS 40S	9.62^{l-q}	10.76^{a-i}	10.96^{a-d}	10.98^{a-c}	**10.58**^{BC}	**10.10**^{B}
	NS Azra	10.32^{b-p}	10.36^{b-p}	10.12^{b-p}	9.84^{h-q}	**10.16**$^{C-E}$	
	NS Ilina	9.92^{f-q}	10.28^{b-p}	10.33^{b-p}	10.26^{b-p}	**10.19**$^{C-E}$	
	NS Petrija	9.15^{q}	9.11^{q}	9.68^{l-q}	9.93^{f-q}	**9.47**^{F}	
Average (F × D)		**9.75**^{D}	**10.13**$^{B-D}$	**10.27**$^{A-C}$	**10.25**$^{A-C}$	-	-
50 N	NS 40S	10.52^{a-m}	10.82^{a-g}	10.77^{a-i}	11.08^{ab}	**10.80**^{AB}	**10.17**^{B}
	NS Azra	9.67^{l-q}	9.81^{h-q}	10.01^{d-q}	10.39^{b-p}	**9.97**^{E}	
	NS Ilina	10.33^{b-p}	10.42^{b-n}	10.45^{a-m}	10.33^{b-p}	**10.39**$^{B-E}$	
	NS Petrija	9.75^{k-q}	9.47^{n-q}	9.42^{pq}	9.54^{m-q}	**9.54**^{F}	
Average (F × D)		**10.07**$^{B-D}$	**10.13**$^{B-D}$	**10.16**$^{B-D}$	**10.34**^{AB}	-	-
100 N	NS 40S	10.79^{a-h}	10.93^{a-e}	11.04^{ab}	11.38^{a}	**11.03**^{A}	**10.39**^{A}
	NS Azra	9.76^{j-q}	10.03^{c-q}	10.54^{a-l}	10.38^{b-p}	**10.18**$^{C-E}$	
	NS Ilina	9.94^{f-q}	10.16^{b-p}	10.38^{b-p}	10.87^{a-f}	**10.34**$^{C-E}$	
	NS Petrija	9.90^{f-q}	10.26^{b-p}	9.85^{g-q}	9.97^{e-q}	**10.00**^{E}	
Average (F × D)		**10.10**$^{B-D}$	**10.35**^{AB}	**10.45**^{AB}	**10.65**^{A}	-	-
150 N	NS 40S	9.80^{i-q}	10.73^{a-j}	10.68^{a-k}	10.95^{a-d}	**10.54**$^{B-D}$	**10.23**^{AB}
	NS Azra	9.62^{l-q}	10.15^{b-p}	10.27^{b-p}	10.41^{b-o}	**10.11**^{DE}	
	NS Ilina	9.44^{o-q}	10.52^{a-l}	9.69^{l-q}	10.33^{b-p}	**10.00**^{E}	
	NS Petrija	10.45^{a-m}	10.48^{a-m}	10.41^{b-n}	9.81^{i-q}	**10.29**$^{C-E}$	
Average (F × D)		**9.83**^{CD}	**10.47**^{AB}	**10.26**$^{A-C}$	**10.37**^{AB}	**Average (C)**	
Average (C × D)	**NS 40S**	**10.18**^{CD}	**10.81**^{AB}	**10.86**^{A}	**11.10**^{A}	**10.74**^{A}	**Grand mean:**
	NS Azra	**9.84**^{D}	**10.09**^{CD}	**10.23**^{CD}	**10.26**^{CD}	**10.10**^{B}	
	NS Ilina	**9.91**^{D}	**10.35**^{C}	**10.21**^{CD}	**10.45**^{BC}	**10.23**^{B}	
	NS Petrija	**9.81**^{D}	**9.83**^{D}	**9.84**^{D}	**9.81**^{D}	**9.82**^{C}	**10.22**
Average (D)		**9.94**^{B}	**10.27**^{A}	**10.29**^{A}	**10.40**^{A}	-	

Different letters represent significant differences (p<0.05; Duncan's multiple range test)

Moreover, cultivars differed significantly in GY, with average values ranged from 9.82 (NS Petrija) to 10.74 t ha^{-1} (NS 40S). Cultivars NS Azra and NS Ilina had a statistically equal GY. Also, there was a significant influence of F×C interaction on GY, indicating that cultivars responded differently to N treatments. Thus, the cultivars NS 40S and NS Azra achieved the highest GY at 100 N, NS Ilina at 50 N and NS Petrija at 150 N. Various studies showed GY increase with nitrogen application as a result of enhanced tillering, higher biomass production and main yield components (Aćin et al., 2019; Jaćimović et al., 2014; Yang et al., 2019). Although, negative influence of N-fertilizer application on GY (severe lodging) were recorded due to favorable conditions for organic matter mineralization and consequently higher mineral N content in the soil (Aćin et al., 2013).

On average for examined cultivars, the highest grain yield was achieved with 900 viable seeds m^{-2} (Tab. 2). However, no statistically significant difference was found among treatments with 900, 700 and 500 viable seeds m^{-2} (10.40, 10.29 and 10.27 t ha^{-1}, respectively). All previously listed sowing densities achieved significantly higher GY compared to the treatment with 300 viable seeds m^{-2} (9.94 t ha^{-1}). In general, GY of most cultivars (except NS Petrija) improved with increasing sowing densities to the highest value, but due to the relatively small contribution of C×D interaction to GY, differences between sowing densities were significant only for cultivars NS 40S and NS Ilina (Tab. 2). Similar results are reported by Aćin et al. (2019) who stated that increase in plant density was followed by increase in GY, without significant differences between 500-900 viable seeds m^{-2}. Moreover, interaction of C×D for GY was significant for only two (of five) analyzed cultivars, with lowest values obtained at 300 viable seeds m^{-2}. Plants may compensate lower population densities by increasing production and survival of tillers and, to a lesser extent, increasing grain numbers per spike (Bokan and Malesevic, 2004). However, although low plant density induces a higher grain number and weight per spike, generally this is not sufficient to compensate for the lower spike density per m^2 generated by a lower tiller density. Therefore, an appropriate increase in plant density to balance yield component factors would appear to be an appropriate management strategy for enhancing wheat GY (Li et al., 2016).

Response of examined wheat cultivars to N-topdressing and sowing density was subjected to regression analysis in order to find optimal values of N in topdressing and optimal sowing density for maximum GY of each cultivar (Fig. 1).

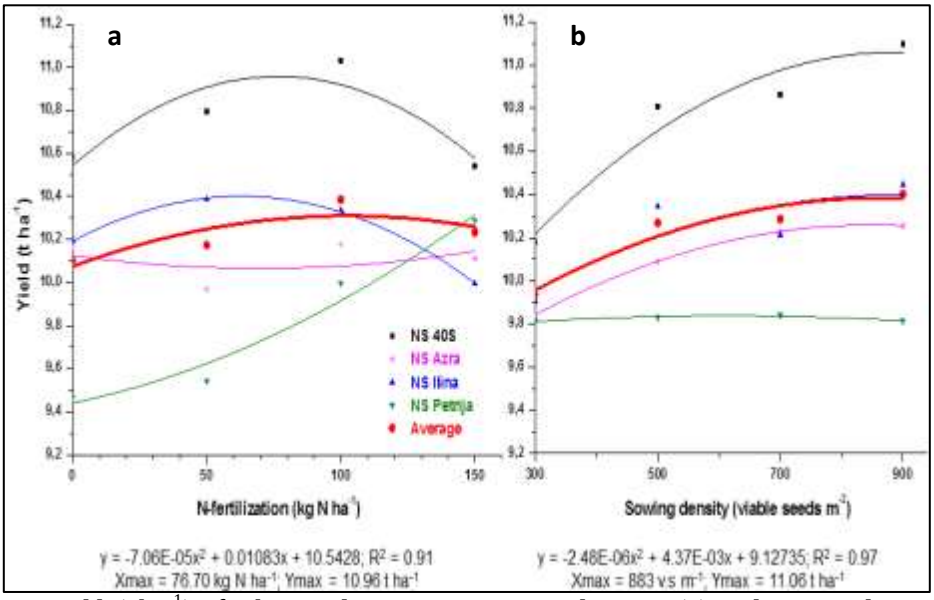


Figure 1. Grain yield (t ha^{-1}) of wheat cultivars across N-topdressing (a) and sowing density treatments (b) and the corresponding regression equations

Effects of the applied N rates in topdressing on GY (on average for all sowing densities) followed the quadratic regression model, for both individual cultivars and average, with different coefficients of determination (Fig 1a). The amounts of N for the theoretical maximum GY varied between cultivars. Thus, for cultivar NS 40S the theoretical maximum GY can be achieved with 77 kg N ha^{-1}, for NS Ilina with 63 kg N ha^{-1}, while the regression curve for cultivar NS Petrija had a constant growth tendency, without possibility to determine the optimal N dose, which also applies to the NS Azra. On average for all cultivars, the theoretical maximum GY (10.31 t ha^{-1}) could be achieved by topdressing with 102 kg N ha^{-1}. Optimal sowing densities also differed between cultivars (Fig 1b). To achieve the maximum yield, the optimal sowing density for cultivar NS 40S was 883 viable seeds m^{-2}, for NS Azra 847, NS Ilina 889 and NS Petrija 610 seeds m^{-2}. On average for cultivars, the optimal sowing density for maximum GY was 862 viable seeds m^{-2}. However, as pointed out above, no significant differences were found between 500, 700 and 900 seeds m^{-2}, and from an economic point of view the optimal sowing density can be considered 500 viable seeds m^{-2}. While the literature reports a wide range of models representing GY as a function of seeding rate, the quadratic is usually the most often reported to represent lodging and other potential yield losses due to increased pressure of insects and diseases at high populations (Fischer et al., 2019). Our results are in agreement with findings of Otteson et al. (2007), who reported that individual genotypes responded differently to varying seeding rates.

Conclusion

The results from this study indicated different cultivar responses to N-topdressing and sowing density treatments. The highest GY was observed with 100 and 150 kg N ha^{-1}, without significant differences between 150, 50 and 0 kg N ha^{-1}. Cultivars differed significantly in GY and the average values ranged from 9.82 to 10.74 t ha^{-1}. Based on the quadratic regression, the contribution of N-topdressing and different sowing densities for maximum grain yield were specific to each cultivar. The maximum GY could be achieved by topdressing with 102 kg N ha^{-1} and with sowing density of 862 viable seeds m^{-2}. However, no significant differences were found between densities of 500-900 viable seeds m^{-2}. Consequently, from an economic point of view the optimal sowing density can be considered 500 viable seeds m^{-2}. The existence of significant interaction between N-fertilization and cultivars as well as cultivars and sowing densities indicate that the adjustment of analyzed agro-management practices to each cultivar is essential for achieving high GY in winter wheat.

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MECHANIZATION OF HEMP HARVEST AND ITS IMPORTANCE

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Abstract

Hemp (*Cannabis sativa* L.) plant components are evaluated in different ways in the industrial and textile fields. There are factors that limit the commercialization of hemp in the agro-industrial chain. From an agricultural point of view, deficiencies in harvesting methods are important among these factors. Often, solutions to these problems in harvesting methods have been tried to be found with local agricultural practices. Harvest time is important in hemp because late harvest should not be done. Delayed harvesting leads to increased sap yield, but to decreased fiber yield and quality in the plant. Therefore, hemp reaching harvest maturity should be harvested as soon as possible. Hemp harvesting is usually done by hand, but in recent years the method of machine harvesting has also been used. Harvesting by hand causes too much labor and time to be wasted. As a result of the surplus labor requirement, the chances of competition against other fibrous plants decreased and the cultivation area decreased in many countries. For this reason, especially machine harvesting has increased its importance day by day. Hemp harvesting has developed on a local basis. However, research is still ongoing on harvesting machine where fiber quality will be maintained and seed loss will be minimal in existing harvesters. In particular, hemp harvesting mechanization focuses on reducing the losses in the mowing patterns of harvesters and increasing labor efficiency. The aim of this paper is to present a review of the existing the current mechanical harvesting systems for Hemp, one of the most efficient fiber plants, and to provide information about the machines that need to be worked on.

Keywords: *Hemp, fiber, mechanization, harvesting, harvester.*

Introduction

Hemp (*Cannabis Sativa* L.) is one of the first important cultural plants in the history of humankind, cultivated for its fiber and seed. Hemp (*Cannabis Sativa* L.) is one of the first important cultural plants in human history to be cultivated for its fiber and seed.

It was the main purpose of humans in ancient times to meet their nourishment, clothing, and shelter needs. In addition to the use of plants in nutrition, the fibers obtained from the stems, leaves, and roots of the plants were used for clothing and shelter. Today it is known that up to two thousand plants produce natural fiber, although only a few are commercially important. Around 90 % of natural fiber production in the world is provided by these plants (Gowda, 2007). Among the important fiber, plants are linen, hemp, cotton, jute.

The whole of the hemp plant parts is utilized in different ways in the industry and textile fields. The hemp plant is used in different fields such as textile, paper, cosmetics, pharmaceuticals, automotive, furniture, nutrition, construction materials. It has also recently gained importance as a renewable raw material for the production of strong, lightweight, composite materials. Due to ecological concerns in humans, the demand for textile products made from hemp fiber is increasing day by day. In addition, the superior performance and utilization properties of hemp fibers due to their physical and chemical structures attract the

attention of all sectors (Özdemir and Tekoğlu, 2013). Hemp fibers now account for less than 0.5% of the world's natural fiber production (Shahzad, 2011). Compared to cotton, which is the most commonly used natural fiber in the world, and petroleum-based synthetic fibers, hemp fibers attract attention in textiles with their organic production possibilities and outstanding environmental characteristics (Acar and Dönmez, 2016). Hemp plants are harvested for fiber or both fiber and seed. There are methods such as mechanical separation, raw soaking, pooling, chemical treatment, and enzyme application to separate the fibers from the stems after a hemp harvest. One of the important parameters that affect the properties of the fiber is maturity. The harvesting period of the plant determines fiber maturity. The harvest period should be selected according to the desired fiber quality (Kaya and Öner, 2020).

Mechanical harvesting is one of the important factors in the agricultural management of fibrous plants. In harvesting, threshing systems are looking for technically easy and practical solutions. Some countries in the world try to develop hemp harvesting solutions according to their local practices.

In the production of hemp, there are soil preparation, planting, intermediate processes management, harvesting, and fiber separation processes. Harvesting hemp stalks constitute the most critical stage of these processes. Considering the hemp production process, labor, and time consumption cover approximately 40 % of the whole process (Huang *et al.*, 2017). For this reason, it is important to develop a harvesting machine in hemp growing. Thanks to the improvements in harvest mechanization, it will be possible to obtain quality fiber.

Sharing existing technologies and innovations in this field in the world will positively affect the developments in harvest systems (Pari *et al.*, 2015).In many countries in the world, researches are carried out in this area. It is also known that there is a need for technological advances in hemp harvest mechanization (Gusovius *et al.*, 2016). From an agricultural point of view, technological deficiencies or gaps in harvesting systems that prevent the full use of crops are important. High quality fiber production for hemp depends on the quality of the raw material. This study consists of a review of existing mechanical harvesting systems applied in hemp plants.

Material and Methods

Thanks to the harvesting and chopping of plants such as hemp, plant material can be processed directly and high-quality fibers can be obtained.

In the harvest of hemp plants, besides hand harvesting, the countries are using their locally developed harvesting machines, according to their own growing conditions and applications. However, machines developed in different countries are not suitable for use in small-scale production areas. Some of the harvesting machines in use, on the other hand, could not find widespread use since they are expensive. Hemp fibre tends to wrap around the rotational machine parts of all types of field equipment. All these characteristics make hemp handling more difficultly than the handling of any other crops. For this reason, the machine is an important need for hemp harvest.

Equipped with special power and the main body of the combined harvester includes handle lifter, cutter, three groups of transverse conveyor, longitudinal force conveyor and picking device, etc. The machine, which can perform cutting and transportation operations at once in industrial hemp harvest (Figure 1), may be a reference to the work to be done (Huang et al., 2017).



Figure 1. Combine prototype harvester

In some countries, the mechanization of hemp is not at the expected level, and generally, researches are carried out on fiber stripping machines (Lu *et al.*, 2013; Zhou *et al.*, 2017). In industrial hemp harvest, the stems are made by cutting from the bottom. These stems are peeled by machines or manpower.

In Turkey, harvesting is done by hand or machine. In harvesting done by hand in small areas, the stems are removed and left to dry (Figure 2a). In the harvests made with the mower, the stems are left to dry after being cut (Figure 2b). One person can harvest about 3 da area per day by hand, while 20-25 da area can be harvested by tractor mower.



(a) (b)

Figure 2. Hemp harvest and drying by hand or tractor mower

Results and Discussion

Harvesting machines developed in countries such as Canada and Germany for use in hemp harvesting (Chen, 2013), have not been widely used as they are not suitable for small field hemp cultivation harvests.

Hemp was harvested to be stored in warehouses for different evaluations (Figure 3). It is important to design the harvesting methods during harvest so as not to impair hemp fiber quality (Idler *et al.*, 2011).



Figure 3. Hemp harvesting machines with different mowing patterns

Hemp should be harvested when the weather conditions are favorable. Especially in humid climates, there is a decrease in fiber yield and quality when the harvest is delayed (Bennet *et al.*, 2006). Under normal conditions, it is left to dry in the field after harvest to obtain fiber. However, due to weather conditions being a problem for harvesting and drying, technological innovations that do not depend very much on weather conditions need to be developed.

In some countries, machines used in the harvesting of plants such as corn are also used in hemp harvesting. These machines, on the other hand, negatively affect the fiber quality as a result of incorrect cutting of the hemp product due to errors in harvesting technology.

The results of the researches for the determination of stem cutting speed in combine harvester design and stalk feeding speed can be applied to new researches to be conducted (Shen *et al.*, 2017). Taking the machines used in the hemp harvest as a reference will contribute to developments in this regard.

It will be useful to consider the current technological developments in order to reduce costs and improve hemp production with hemp mechanization applications (Amaducci *et al.*, 2015). During the development of the hemp harvesting machine, it is also important to examine the physical and mechanical properties of hemp (Shen et al., 2015).

Some mechanical parameters of cannabis such as tensile tests, pressure tests, and bending tests are also taken into consideration in the design of hemp harvesting machines. (Yang et al., 2016). Generally, hemp for fiber production is harvested at full flowering of male plants. This harvest the maximum fiber is obtained. Although there are different harvesters used for hemp harvest, precision harvesters are needed for high fiber quality. Harvest mechanization should be developed to obtain hemp quality fiber.

Conclusions

In recent years, cultivation and harvesting procedures that provide the supply of fibrous raw materials for technical products are developed preferentially.

Although there are improvements in the harvesting machines used for hemp harvesting, some changes are still needed due to the specific properties of hemp. Especially long stems of hemp plants or loose fibers cause windings in the harvesting machine. Developments should be made to prevent these damages caused by winding.

Mechanization applications are extremely important in harvesting in order to obtain quality fiber or seeds from hemp. For this purpose, researches should be continued to develop suitable and economical harvesting machines, considering the specific characteristics and growing conditions of hemp plants. More research needs to be done on the combined harvester, especially for fiber and seed harvesting.

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THE INFLUENCE OF VARIOUS SOIL TILLAGE SYSTEMS ON ENERGY CONSUMPTION IN WINTER WHEAT AND RYE PRODUCTION

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Abstract

The production of small grains can be obtained by using various soil tillage systems. Although the conventional tillage demands the highest energy inputs with respect to other systems, it is still the most commonly used tillage system in Serbia. An inadequate tillage system reduces yield and increases production costs. The paper presents the results of examining the impact of different tillage systems on energy consumption in the production of winter wheat and rye, as well as the yields obtained. The tests were performed in the vicinity of Vršac on degraded chernozem type soil, and included three tillage systems: conventional tillage system - CT, conservation tillage system - RT and the variant without tillage, i.e. direct sowing - NT. The obtained results showed that in the production of winter wheat using the conventional tillage system - CT measured the highest energy consumption which averaged 66.43 [l ha^{-1}] or 2570.84 [MJ ha^{-1}]. In the variant of direct sowing - NT, we measured significantly lower values of energy consumption compared to the conventional tillage system, on average 11.17 [l ha^{-1}] or 432.28 [MJ ha^{-1}]. By applying conservation tillage, an average of 51.41 [l ha^{-1}] and 1989.57 [MJ ha^{-1}] were spent, respectively. A similar impact of the applied tillage system on energy consumption was observed in the production of winter rye. The highest yield of winter wheat was achieved in the variant of direct sowing NT, and the lowest in the variant of conservation treatment RT, while in the production of winter rye the highest yield was achieved in the variant of conservation treatment RT, and the lowest in the variant of conventional treatment CT.

Keywords: *Tillage, Energy consumption, Yield, Wheat, Rye.*

Introduction

One of the basic operations in agricultural production, which aims to create favorable conditions for the plant, is tillage. Winter wheat and rye can be produced using different tillage systems, and the most common system in the production of these crops is conventional tillage. By applying the system of conventional tillage after plowing, it is often the case that a disc harrow is used to shred lumps and harvest residues several times, which significantly affects the soil structure, increases the risk of erosion, higher energy consumption and production costs (Javadi *et al.*, 2006). Choosing the wrong tillage system adversely affects moisture retention, yield and production costs (Boydas *et al.*, 2007; Sarauskis *et al.*, 2009). A good substitute for the classic tillage is conservation tillage due to a number of advantages that are especially emphasized on winter crops sowing (Kovačević *et al.*, 2005; Rusu *et al.*, 2013). Tillage systems determine soil properties (Tabaglio *et al.*, 2008; Aziz *et al.*, 2013), water-air balance and consequently crop yield and its quality (Ruisi *et al.*, 2014; Montemurro *et al.*, 2015; Rachon *et al.*, 2015; Wozniak *et al.*, 2017). It is possible to significantly reduce energy consumption and increase the profitability of production by applying minimal land cultivation (Failla *et al.*, 2020). The choice of the optimal tillage system enables the creation

of the best conditions for growth, development and yields. Compared to the conventional system, in the conservation system, fuel consumption is lower by 27.4%, and in the zero processing system NT as much as 82.7%. The highest yield of winter wheat of 5.73 t ha^{-1} was achieved in the conservation tillage system, while in the variant of conventional tillage the yield was 5.22 t ha^{-1}, and in the variant of direct sowing - NT 5.62 t ha^{-1} (Filipović *et al.*, 2004). In conventional tillage - CT, fuel consumption was 67.72 l ha^{-1}, while in the RT system consumption was lower by 21.6%, and in the variant without tillage - NT even by 90.1% compared to conventional tillage - CT system (Košutić *et al.*, 2008). Winter wheat grain yield achieved by reduced tillage systems was in average either higher CH=5.59 t ha^{-1} or not different 5.38 and 5.23 t ha^{-1} for DS and NT, respectively than CT 5.28 t ha^{-1} (Jug *et al.*, 2006). The yield of winter wheat was 6.79 t ha^{-1} by applying the system of zero tillage - NT, and in the application of conventional tillage - CT 6.49 t ha^{-1} (Košutić *et al.*, 2006). Lower yields of winter rye were achieved in the variant of conventional tillage - CT compared to reduced tillage - RT by 23.10% (Barać *et al.*, 2013). A higher barley grain yield was determined in CT system compared to RT system (by 24.8%) and NT (by 54.2%) systems (Wozniak, 2020). The aim of the study was to determine the effects of work and the impact of the applied tillage system on energy consumption and yield of winter wheat and rye in the examined conditions.

Material and Methods

Investigations of the impact of different tillage systems on energy consumption and yield of winter wheat and rye were performed in the production conditions of 2018/19. in the vicinity of Vršac (South Banat in Serbia) 45°07'10.9"N 21°07'07.8"E on soil type degraded chernozem according to FAO classification. Winter wheat of the Pobeda variety and winter rye of the Raša variety were used for sowing. The tests covered three different tillage systems. The first tillage system was conventional tillage - CT as the most common system. Within this system, plowing was performed with a furrowing plough, then shredding with disc harrow (2 pases), pre-sowing preparation with a seed drill, after which sowing was performed. The second tillage system was the conservation tillage - RT, based on the primary application of a chisel plough, followed by the two subsequent passes of a disc harrow, after which the sowing was performed. Conventional soil tillage was performed at a depth 20 - 22 cm, while the conservation tillage was performed at a depth of 12 - 15 cm. The third system was direct sowing, i.e. a variant without processing - NT. Tractors equipped with 46.5 kW, or 64.5 kW diesel engines were used to tow the attachments used in the tested tillage systems. The sowing norm was 240 kg ha^{-1} for winter wheat, or 180 kg ha^{-1} for a winter rye, which provided about 600 germinating grains of winter wheat per m^2, or 550 germinating grains per m^2 of the winter rye. The tests included analysis the impact of different tillage systems on fuel consumption, energy, labour productivity and the amount of yields.

Table 1. Technical characteristics of the tested machinery

Parameters	Working tool						
	Unit	Furrowing plough	Disc Harrow Sava 24	Seed beder Agromerkur SS 2.10	Chisel plough Uzel 200	Seeder IMT 634.23	No-till seeder Anzek H HMA 24
Work width	[cm]	70	210	210	200	276	350
Depth of tillage	[cm]	up to 30	up to 12	up to 15	30-40	up to 10	5-12
Mass	[kg]	290	420	300	450	501	3100
Required tractor power	[kW]	44	26-33	30	40-50	26	55

Table 1 shows technical characteristics of the tested machinery used in the tested tillage systems. Fuel consumption in our research was measured by the volumetric, "top-up" method (Moitzi et al., 2013), and labor productivity by chronometer. The realized yields were determined on the diagonal of the plot at the time of harvest and were calculated for the entire experiment, and all values were in 5 replications. The experiment was performed in a completely random plan, and the obtained results were processed using Microsoft Office Excel 2007.

Results and Discussion

Table 2 and 3 shows the results of examining the impact of tillage systems on energy consumption and the amount of realized yields of winter wheat and rye. The obtained results show that there are significant differences between the examined tillage systems in terms of fuel consumption, i.e. energy, as well as the amount of realized yields.

As it was expected, the highest average fuel consumptions were realized in the variant of conventional tillage - CT: 66.43 [l ha^{-1}] in the production of winter wheat, and 64.22 [l ha^{-1}] in the production of winter rye. When the system of conservation tillage – RT was applied, the average fuel consumption in the production of winter wheat was 51.41 [l ha^{-1}], while in the production of winter rye in this tillage system the average consumption was 52.13 [l ha^{-1}]. The lowest average fuel consumption of 11.17 [l ha^{-1}] was achieved in the variant without processing NT- direct winter wheat sowing, while in the no till production of winter rye an average of 11.68 [l ha^{-1}] was used (Table 2).

Table 2. Energy consumption and realized yields depending on the tillage system

Tillage System	Winter wheat Cropping period 2018/2019			Winter rye Cropping period 2018/2019		
	Average fuel consumption [l ha^{-1}]	Average energy requirement [MJ ha^{-1}]	Average efficiency [ha h^{-1}]	Average fuel consumption [l ha^{-1}]	Average energy requirement [MJ ha^{-1}]	Average efficiency [ha h^{-1}]
Conventional tillage (CT)						
Plough	31.95	1236.46	0.25	31.56	1221.37	0.23
Disc Harrow 2 x	20.45	791.42	0.87	18.23	705.50	0.94
Seed-Bed preparing	6.93	268.19	1.31	7.11	275.16	1.40
Sowing	7.10	274.77	1.36	7.32	283.28	1.47
Total	66.43	2570.84	/	64.22	2485.31	/
Conservation tillage (RT)						
Chisel	22.19	858.75	0.65	20.75	803.03	0.70
Disc Harrow 2 x	21.53	833.21	1.01	24.17	935.38	0.97
Sowing	7.69	297.60	1.48	7.21	279.02	1.57
Total	51.41	1989.57	/	52.13	2017.43	/
No till (NT)						
No till planter	11.17	432.28	1.50	11.68	452.02	1.54

Analyzing the influence of the applied tilling systems on energy consumption, it was noticed that the chosen tilling system had a strong impact on the level of energy consumption in the production of both crops. Thus, by applying conventional tillage - CT in the production of

winter wheat, an average consumption was 2570.84 [MJ ha^{-1}], i.e. 2485.31 [MJ ha^{-1}] in the production of winter rye. By applying conservation tillage - RT, lower energy consumption was achieved compared to conventional tillage and in the production of winter wheat it was 1989.57 [MJ ha^{-1}], while in the production of winter rye it was 2017.43 [MJ ha^{-1}]. By applying direct sowing, i.e. the variant without processing - NT, the lowest energy consumption was obtained, which in the production of winter wheat amounted to 432.28 [MJ ha^{-1}], while in the production of winter rye the energy consumption was 452.02 [MJ ha^{-1}] (Table 2).

Comparing fuel consumption between the examined tillage systems, it is noticed that the application of direct sowing - NT in the production of winter wheat provides the opportunity to achieve large savings in energy consumption, 83.19% compared to conventional tillage system - CT, or 78.27% compared to conservation tillage - RT. A similar influence of the examined tillage system on fuel consumption is observed in the production of winter rye.

Other authors have obtained similar results in their research (Filipović *et al.*, 2004; Kovačević *et al.*, 2005; Javadi *et al.*, 2006; Košutić *et al.*, 2008; Sarauskis *et al.*, 2009; Rusu *et al.*, 2013; Failla *et al.*, 2020).

Table 3 presents basic descriptive statistical parameters, which illustrate the influence of tillage system on the yield of winter wheat and winter rye.

Table 3. Influence of tillage system on the yield of winter wheat and winter rye – basic descriptive statistics

Tillage System	Winter wheat yield [t ha^{-1}]					Winter rye yield [t ha^{-1}]				
	Mean	σ	Cv	Min	Max	Mean	σ	Cv	Min	Max
Conventional tillage (CT)	4.71	0.24	5.05	4.38	4.97	2.14	0.11	4.96	1.98	2.26
Conservation tillage (RT)	4.52	0.24	5.26	4.27	4.89	2.58	0.15	5.83	2.39	2.77
No till (NT)	5.11	0.13	2.63	4.92	5.28	2.31	0.14	6.00	2.14	2.46

Based on the results shown in Table 3, it can be observed that the applied tillage system significantly affected the level of yields in both crops. Thus, the highest yield of winter wheat was achieved in the variant of direct sowing - NT and amounted to 5.11 [t ha^{-1}] and the lowest in the variant of conservation treatment - RT, 4.52 [t ha^{-1}], while in the variant of conventional tillage - CT achieved an average yield of 4.71 [t ha^{-1}].

In the production of winter rye, the highest yield of 2.58 [t ha^{-1}] was achieved in the variant of conservation tillage - RT, and the lowest in the variant of conventional tillage - CT, 2.14 [t ha^{-1}], while by direct sowing - NT achieved an average winter rye yield of 2.31 [t ha^{-1}] (Table 3).

Similar results are reported by other authors in their research (Boydas et al., 2007; Sarauskis et al., 2009; Filipović et al., 2004; Jug et al., 2006; Košutić et al., 2006; Barać et al., 2013; Wozniak, 2020).

Conclusions

Based on the obtained results, it can be concluded that the examined tillage systems had a significant impact on fuel and energy consumption. The highest fuel consumption was measured in the variant of conventional tillage - CT and amounted to 66.43 [l ha^{-1}] in the production of winter wheat and 64.22 [l ha^{-1}] in the production of winter rye. By applying conservation tillage - RT in the production of winter wheat, the average fuel consumption was 51.41 [l ha^{-1}], while in the production of winter rye in this tillage system, an average of 52.13 [l ha^{-1}] was used. The lowest average fuel consumption of 11.17 [l ha^{-1}] was achieved in the variant without processing - direct sowing - NT in the production of winter wheat, while in

the production of winter rye an average of 11.68 [l ha^{-1}] was used. By applying conventional tillage - CT, the highest energy consumption was achieved in the production of winter wheat 2570.84 [MJ ha^{-1}], or 2485.31 [MJ ha^{-1}] in the production of winter rye, while by applying direct sowing, i.e. the variant without cultivation - NT the lowest energy consumption was realized, which in the production of winter wheat averaged 432.28 [MJ ha^{-1}] and 452.02 [MJ ha^{-1}] in the production of winter rye.

The application of direct sowing - NT in the production of winter wheat resulted in 83.19% lower energy consumption compared to the conventional tillage system - CT, or 78.27% compared to the conservation system - RT. A similar influence of the examined tillage system on energy consumption is observed in the production of winter rye. The highest yield of winter wheat was achieved in the variant of direct sowing - NT and amounted to 5.11 [t ha^{-1}] and the lowest in the variant of conservation treatment - RT, namely 4.52 [t ha^{-1}]. In the production of winter rye, the highest yield of 2.58 [t ha^{-1}] was achieved in the variant of conservation tillage - RT, and the lowest in the variant of conventional tillage - CT, namely 2.14 [t ha^{-1}].

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INFLUENCE OF INTERCROPPING SWEET SORGHUM WITH SOYBEAN ON FORAGE YIELD AND QUALITY

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Abstract

Cereals are highly important in feeding ruminant animals for their high dry matter production and low cost. Sweet sorghum is an important silage crop and has an increasing popularity because of the need for relatively smaller quantities water per unit dry matter production of maize. Regarding high feed costs of protein supplementations, legumes can be used in livestock nutrition for their high protein content, thus, providing cost savings. Since legumes have low dry matter yield, acceptable forage yield and quality can be obtained from intercropping cereals and legumes compared with their sole crops. Sweet sorghum and soybean intercropped in various row ratios and pure sweet sorghum crop were evaluated to determine the best intercropping system with respect to forage yield and quality. The highest dry matter yield over two years (20.6 t ha^{-1}) was produced by intercropping 1 row of sweet sorghum with 3 rows of soybean, whereas the lowest yield (17.9 t ha^{-1}) was produced with intercrop consisting of 1 row of sweet sorghum with 1 row of soybean. All intercropp had higher crude protein values in dry matter averaging 105 g kg^{-1} for the 1SB1S, 116 g kg^{-1} for the 1SB2S and 128 g kg^{-1} for the 1SB3S, than the monocrop of sweet sorghum (72 g kg^{-1}). Intercropping of sweet sorghum with soybean reduced neutral detergent fiber content, which in turn, resulted in increased forage digestibility. Based on forage yield and quality, this study showed that among all intercropped forages the 1SB3S treatment was better performing than other intercrops.

Keywords: *Intercropping, Sweet Sorghum, Soybean, Yield, Forage Quality*

Introduction

Intercropping is the practice of cultivating two or more crops simultaneously on the same piece of land during the same time span (Guleria and Kumar, 2016). It is characterized by rotation and diversification in time and space dimensions (Biabani et al., 2008). Intercropping systems help farmers to exploit the principle of diversity (Ghosh, 2004). They help avoid reliance on a single crop and result in different products of different nature such as fodder, oil and pulses (Iqbal et al., 2018a). Another key benefit associated with intercropping is its potential to increase soil productivity per unit area and efficient utilization of farm resources (Mucheru-Muna et al., 2010). Cereals intercropping with legumes result in increased resource capture by component crops and improve soil microbial activity along with improved conversion resource efficiency that drives higher biomass production (Alvey et al., 2003). In addition, soil fertility improves when legumes are intercropped with cereal forages (Iqbal et al., 2018b).Intercropping of cereals with legumes also increases productivity per unit of land area due to atmospheric biological nitrogen fixation (BNF) that takes place in the root nodules of legumes (Pal and Sheshu, 2001). Greater productivity per unit of surface area of sorghum-soybean intercropping systems resulted in 46% higher yields than their monocrop sorghum

(Iqbal et al., 2017). Sorghum is an important silage crop and has an increasing popularity because of the need for relatively smaller quantities of maize water per unit dry matter production (Bean et al., 2013). Legumes contain more than twice as crude protein than forage sorghum, therefore, sorghum-legume intercropping has the potential to increase the biomass and quality of forage per area compare to sole sorghum (Eskendari et al., 2009). Forage sorghum can be intercropped with forage legumes such as cowpea cluster bean, soybean etc. which are totally compatible with sorghum in terms of sowing time and irrigation (Iqbal et al., 2015). The present study was designed to determine the influence of different patterns of sweet sorghum-soybean intercropping on forage yield and quality.

Material and Methods

A field experiment was carried out during the 2016 and 2017 growing season at experimental fields in Daruvar (45°35'34″N, 17°13'25″E), Croatia. Meteorological data of the experimental site are presented in Table 1.

Table 1. Monthly meteorological data during the growing seasons in 2016 and 2017

Year	Meteorological data	Month					
		April	May	June	July	August	September
2016	Mean air temperature (°C)	12.3	15.5	20.4	22.3	19.4	17.0
	Rainfall (mm)	35.3	83.7	99.6	152.6	66.2	48.5
2017	Mean air temperature (°C)	10.9	16.5	21.8	22.9	22.4	14.7
	Rainfall (mm)	62.8	45.0	70.3	71.9	29.0	121.7

The experimental design was set up a randomized complete block system with three replicates. Sweet sorghum was seeded as sole crop (SB) and intercropped with soybean as follows: 1 sweet sorghum row to 1 soybean row (1SB1S), 1 sweet sorghum row to 2 soybean rows (1SB2S) and 1 row sweet sorghum to 3 rows soybean (1SB3S). Sorghum hybrid seed "KWS Zerberus" was obtained from Seed Company „KWS". The soybean cultivar seed known as „OAC Wallace" was obtained from „RWA Agro" company. The individual plot size was 50 m × 2.8 m for each treatment. The sweet sorghum and soybean were spaced at 70 cm × 5.7 cm with population of 250 000 sweet sorghum and 250 000 plants soybean per hectare, respectively. Tillage was carried out in autumn by ploughing to 30 cm depth. Presowing seedbed preparation was done in spring using a tractor-mounted rototiller. All plots were fertilized with the same amount of fertilizer before sowing, containing 150 kg of N ha^{-1}, 100 kg P_2O_5 ha^{-1} and 200 kg of K_2O ha^{-1}. Sweet sorghum and soybean were sown to a depth of approximately 5 cm by drill on April 30, 2016 and on April 29, 2017. Herbicide Dual Gold 960 EC (S-metolachlor, 960 g/L a.i.) was applied pre-emergence at a dose of 1 L ha^{-1} and herbicide Basagran 480 (bentazone, 480 g/L a.i.) was applied post-emergence in intercropping sweet sorghum with soybean at a dose of 2 L ha^{-1}. The soil of the research area has a pH 4.4 (M-KCl), 3.3% humus (organic matter), and is poorly supplied with physiologically active phosphorous (7.6 mg P_2O_5/100 g soil), medium supplied with physiologically active potassium (22.3 mg K_2O/100 g soil), while the total nitrogen content averaged 0.15% in topsoil. The fresh fodders were hand harvested when the sweet sorghum reached early hard dough stage while soybean was at R7 stage and then chopped into 10 mm size pieces with a chaff cutter. The dry matter content was determined by drying in an oven at a temperature of 65°C to a constant mass. Crude protein content was measured according to Kjeldahl (AOAC, 2000), neutral detergent fibre according to Van Soest et al. (1991), calcium

were analysed by atomic absorption spectrophotometry by analyzer Spectrophotometer 2010 Model M530 Infrared Spectrophotometer (USA) and phosphorus was analysed by colorimetry (AOAC, 2000). Analyses of variance were made for dry matter yield and forage quality parameters ($P<0.05$), and the Tukey test was used for comparing means ($P<0.05$). Data were analyzed using SAS statistical software (SAS Inst., 2013).

Results and Discussion

The differences in the yield of dry matter (Table 2) were statistically significant ($P<0.05$). Dry matter yields ranged from 17.0 t ha^{-1} (1SB1S) to 19.9 t ha^{-1} (SB) in 2016. In the following growing season of 2017, the yield of dry matter ranged from 18.8 t ha^{-1} (1SB1S) to 22.5 t ha^{-1} (SB). Consequently, dry matter yields were higher in 2017 than in 2016. This could be due to the impact of more favorable environmental factors (effect of the year) such as water and temperature during plant vegetation. The average yield of dry matter over the two years showed that 1SB3S was the best intercropping production system with relatively small yield reduction compared to sole crop sweet sorghum (Table 2). According to obtained results, when the number of soybean rows increased in intercrops, dry matter yields on parcels increased. One of the possible explanations for higher yields for the intercrops is their ability to exploit different layers of soil without mutual competition. Besides, higher consumption of environmental resources, agronomic practices, crop genotypes, photosynthetic active radiation and soil moisture during the rainy season may affect yield and potential use of the intercropping system (Anil et al., 1998; Lithourgidis et al., 2006). Terzić et al., (2004) and Basaran et al., (2017) indicated that, legumes contribution to sweet sorghum in mixtures was significant and increased the dry matter yield. In this study it was found that the yield of crude proteins of intercropped fodder 1SB1S, 1SB2S and 1SB3S was significantly ($P<0.05$) higher than SB (sole crop of sweet sorghum) during a two year study (Table 2). Treatment 1SB3S had the highest yield of crude protein averaging 2.37 t ha^{-1} in 2016 and 2.92 t ha^{-1} in 2017 in comparison to other fodder mixtures (Table 2). According to obtained results, when the number of soybean rows increased in intercrops, crude proteins yields on parcels increased. Terzić et al., (2004) and Basaran et al., (2017) indicated that, legumes contribution to sweet sorghum in mixtures was significant and increased the yield of crude proteins.

Table 2. Yield of dry matter and yield of crude proteins of sweet sorghum and sweet sorghum-soybean intercropped

Treatments	Yield of dry matter in t ha^{-1}			Yield of crude proteins in t ha^{-1}		
	2016	2017	Mean	2016	2017	Mean
SB	19.9a	22.5a	21.2a	1.37d	1.69d	1.53d
1SB1S	17.0c	18.8c	17.9b	1.72c	2.05c	1.89c
1SB2S	18.3bc	20.7b	19.5ab	2.03b	2.50b	2.27b
1SB3S	19.4ab	21.8ab	20.6a	2.37a	2.92a	2.65a
Mean	**18.7b**	**21.0a**		**1.87b**	**2.29a**	

Different letters in the column indicate significant difference ($P<0.05$)

One of the main reasons of intercropping sweet sorghum and soybean is the increase crude protein level in silage. Crude proteins are very important in cattle feed and, silage containing more crude proteins is desirable. In this study it was found that the value of crude proteins of intercropped fodder 1SB1S, 1SB2S and 1SB3S was significantly ($P<0.05$) higher compared to SB (sole crop of sweet sorghum) during a two year research (Table 3).

Table 3. Content of crude protein and content of neutral detergent fiber of sweet sorghum and sweet sorghum-soybean intercropped

Treatments	Crude protein in g kg^{-1} dry matter			Neutral detergent fiber in g kg^{-1} dry matter		
	2016	2017	Mean	2016	2017	Mean
SB	69d	75c	72d	569a	551a	560a
1SB1S	101c	109b	105c	528b	512b	520b
1SB2S	111b	121ab	116b	503c	487c	495c
1SB3S	122a	134a	128a	478d	462d	470d
Mean	**101b**	**110a**		**520a**	**503b**	

Different letters in the column indicate significant difference (P<0.05)

According to obtained results, when the number of soybean rows increased in intercrops, the content of crude protein in the mixture increased. The findings in this study are consistent with other research in which legumes also increased the concentration of crude proteins when grown in mixture with sweet sorghum (Terzić et al., 2004; Basaran et al., 2017). Fodder produced in sweet sorghum-soybean intercrops is important not only because of an increase in the content of crude protein, but also because of reduction the content of neutral detergent fibers. For this reason, the best option in sweet sorghum-soybean intercropping is the use of soybean genotypes that provide forage with the greatest proportion of pods at harvest. In this study it was found that the neutral detergent fibers of intercropped 1SB1S, 1SB2S and 1SB3S were significantly (P<0.05) lower than SB (sole crop of sweet sorghum) during two years of research (Table 3). According to obtained results, when the number of soybean rows increased in intercrops, the values of neutral detergent fibers in the mixture decreased. The content of neutral detergent fiber is important in ration formulation because it reflects the amount of animal forage that animals can consume (Lithourgidis et al., 2006). In general, the concentration of neutral detergent fibers is higher for grass than for legumes (Dahmardeh et al., 2009). Many researchers stated that the nutritional value of cell wall components decreased with plant age and is related to increased lignin content (Atis et al. 2012; Zhao et al., 2012). Since smaller amounts of fiber components are used for better digestion, the soybean intercropped plots to be superior to monocrop sweet sorghum in terms of neutral detergent fiber.

Table 4. Content of calcium and content of phosphorus of sweet sorghum and sweet sorghum-soybean intercropped

Treatments	Calcium in g kg^{-1} dry matter			Phosphorus in g kg^{-1} dry matter		
	2016	2017	Mean	2016	2017	Mean
SB	4.4d	3.6d	4.0d	2.6d	3.0c	2.8c
1SB1S	5.1c	4.1c	4.5c	2.7c	3.1bc	2.9bc
1SB2S	5.8b	4.6b	5.2b	2.8b	3.2ab	3.0ab
1SB3S	6.4a	5.0a	5.7a	2.9a	3.3a	3.1a
Mean	**5.43a**	**4.33b**		**2.75b**	**3.15a**	

Different letters in the column indicate significant difference (P<0.05)

Contreras-Govea et al. (2011) ensiled corn and forage sorghum with different proportions of lablab bean and reported that legume must make up at least 50% of the mixture to affect

fermentation and nutritive value. In this paper, the value of calcium and phosphorus of intercropped forage 1SB1S, 1SB2S and 1SB3S was statistically significantly ($P<0.05$) higher than sole crop of sweet sorghum during a two year study (Table 4). According to obtained results, when the number of soybean rows increased in intercrops, the values of calcium and phosphorus in the mixture increased. Contribution of legumes with sweet sorghum in mixtures was significant and increased potassium, phosphorus, calcium and magnesium in fresh fodder (Terzić et al., 2004; Basaran et al., 2017). Juknevičius and Sabienė (2007) reported that mineral element content in the plants depend on species and families of plant; leguminous plants accumulated more calcium and magnesium than cereal or grasses (*Poaceae*).

Conclusion

The intercropping of sweet sorghum with soybean at different planting patterns was shown to be an effective way to affect dry matter yield and crude protein yield, which in turn, enhanced the nutrient quality of fresh fodder mixture. Intercropping of sweet sorghum with soybean has increased the valueş of crude protein, calcium, phosphorus and decreased values of neutral detergent fiber in fresh fodder mixture. Finally, the 1SB3S treatment (intercropping 1 row of sweet sorghum with 3 rows of soybean) was most effective regarding the nutrition composition in fresh forage.

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INFLUENCE OF LOW TEMPERATURES ON FREEZING OF WINTER BUDS OF MERLOT AND CABERNET FRANC GRAPE VARIETIES

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Abstract

In agro-ecological conditions of the experimental field of the Faculty of Agriculture in Zemun "Radmilovac" – Vinča (Serbia), and in the conditions of the cold storage, the influence of low temperatures during hibernation on the percentage of freezing of winter buds in Merlot and Cabernet Franc varieties of grapes were studied. The aim of the study was to examine the resistance of Merlot and Cabernet Franc varieties to low negative temperatures, during four years of observation. Merlot and Cabernet Franc are quite widespread grape varieties in the agro-ecological conditions of Serbia. Therefore, we believe that it is important to establish their sensitivity to low and negative winter temperatures during hibernation. The in vitro method of artificial freezing of cuttings of one-year-old shoots in the cold storage was applied. Tests were performed simultaneously on both varieties, three times during the winter: December 15, January 15 and February 15, at two different temperatures -20ºC and -25ºC. The conditions in the cold storage were identical to the external conditions. The highest frost resistance of both grape varieties was shown in January, while the highest percentage of frozen winter buds was recorded in December. Damage of winter buds was lower at -20ºC than at -25ºC. Based on the three-way ANOVA, it was found that there were statistically significant differences between varieties, test temperatures, test dates, but also their interaction. However, further analysis for all observed years showed that at a lower temperature, -25◦C, there was no statistically significant difference between varieties in terms of their sensitivity.

Keywords: *grapes, freezing, winter buds, low temperatures, Merlot, Cabernet Franc.*

Introduction

Regular grape production largely depends on low winter temperatures. However, not all grape varieties are equally resistant to low winter temperatures. Differences between varieties are a consequence of genetic factors. They also depend on the method of cultivation and climatic factors during the growing season, particularly during the winter dormancy. Low winter temperatures are a potential danger (when they occur) over a period of several months. Some grape varieties have different resistance to low winter temperatures in the period December - February. The biochemical and physiological processes that take place in a vine during the growing season greatly affect the degree of resistance of the grapevine to low winter temperatures. Merlot and Cabernet Franc varieties are quite widespread red grape varieties in Serbian viticulture.

Low temperatures (frost) are a limiting ecological factor for successful grapevine growing (Jones, 2010). Low winter temperatures that are usual in the period December - February are particularly harmful. On the other hand, autumn or late spring frosts are less harmful and damaging. Proper maturation of annual shoots during the growing season (particularly in autumn months) affects the resistance of grapevine to low winter temperatures (Jackson, 2008).

Exposure of the terrain (vineyard), choice of a variety, cultivation methods and regular agro-technical measures applied in a vineyard can significantly increase the resistance of the grapevine to low winter temperatures. Varieties that open buds later in the spring and have high regeneration capacity after freezing are more resistant to low winter temperatures (Nakalamić and Marković, 2009). Frequent and long-lasting frosts - low temperatures can cause significant damage to the buds, which subsequently affects the yield and quality of grape and wine (Ribereau Gayon et al., 2006; Lisek, 2009).
The aim of this research is to establish the resistance of new varieties of grapes Merlot and Cabernet Franc to low temperatures. These varieties are in the final phase of testing and before the recognition and introduction to the variety list of the Republic of Serbia, in the near future. Based on the obtained and determined test results, it will be possible to give a recommendation in which agroecological conditions of Serbia it will be possible to grow these varieties.

Material and Methods

The experiment was carried out in the period 2011-2015 in the vineyard of the experimental field of the Faculty of Agriculture "Radmilovac" in Vinča (Serbia). The vineyard with the Merlot and Cabernet Franc varieties was established according to the single Guyot system with a distance of 3 meters between the rows and 1 m between the vines in a raw.
The in vitro method of artificial freezing in a cold chamber was used to test the resistance of winter buds to low winter temperatures (Mills et al., 2006; Guzun et al., 1972; Eifert, 1975; Pogosjan, 1975).
Two cuttings of one-year-old shoots with 15 winter buds (a total of 30 winter buds) were sampled at three points: December 25, January 25 and February 25. Cuttings with winter buds were first kept at the temperature of -5ºC for 24 hours. Then, the temperature in the chamber was gradually lowered by 3ºC per hour to a final temperature of –25ºC. Samples (cuttings) were stored at that temperature for 12 hours. The temperature was then gradually increased by 3ºC per hour until a room temperature of 22ºC was reached. The cuttings were then left at room temperature for five days. After that, a longitudinal section of the winter buds was made and a magnifying glass used to establish the degree of damage done to the winter buds. The resistance of winter buds is not same at all points of winter dormancy, so the test is performed at three: at the beginning, middle and end of winter (December 25, January 25 and February 25). Both varieties and their cuttings were treated in the same way to obtain the most reliable results (Lisek, 2012).
The analysis of the data was performed using the ANOVA statistical method available in statistical package SPSS. Since the research addressed three factors: variety, date of measurement and freezing temperature with five repetitions, the model of the three-way ANOVA was used with fixed levels of all observed factors. This model is represented as follows (Snedecor and Cochran, 1989):

$$\boldsymbol{X_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \varepsilon_{ijkl}} \qquad \textbf{(1)}$$

where α_i, $i=1,2$ represents the effect of a variety; β_j, $j=1,2$ represents the effect of the temperature to which cuttings of one-year-old shoots are exposed; γ_k, $k=1,2,3$ represents the effect of point of observation during winter; $(\alpha\beta)_{ij}, (\alpha\gamma)_{ik}, (\beta\gamma)_{jk}$ is the effect of first-order interactions between the observed factors; $(\alpha\beta\gamma)_{ijk}$ is the effect of the second-order interaction and finally ε_{ijkl} is a random error of the model; while the number of repetitions is five which makes $l = 1,2,3,4,5$.

The differences between the observation points during the winter were tested using the LSD test. Since the remaining two factors: variety and temperature have two levels each, the LSD test was not applied. Results of testing the differences between the levels of these two factors are included in the ANOVA table. All tests were performed at a significance level of 0.05.

Results and Discussion

Based on calculated statistical indicators for the variable *percentage of frozen winter buds*, it is possible to draw interesting conclusions (Table 1). Both the Merlot and Cabernet Franc varieties react similarly to low winter temperatures. Over the years of observation, both varieties had the lowest percentage of frozen winter buds in January regardless of the temperature. On the other hand, the highest percentage of frozen winter buds was recorded in December. This is confirmed by the average percentage of frozen winter buds given in column $\bar{x}$. Group of authors, Pržić et al. (2018) came to approximately the same result. They conducted a similar study by observing two other varieties of grape, Cabernet Sauvignon and Sauvignon Blanc, at three low winter temperatures (-15ºC, -20ºC, -25ºC) in three winter months (15 December, 15 January, 15 February) for two years. The observed varieties showed high resistance to low temperatures in January, but the highest percentage of frozen winter buds was recorded in February.

Table 1: Statistical indicators of percentage of frozen winter buds of Merlot and Cabernet Franc varieties for the period 2011-2015

Temperature	Date	2011/2012			2012/2013			2013/2014			2014/2015		
		min	max	$\bar{x}$	Min	max	$\bar{x}$	min	max	$\bar{x}$	min	max	$\bar{x}$
		MERLOT											
-20ºC	15.12.	49	57	53	51	61	56	45	53	49	46	56	51
	15.01.	27	33	30	30	38	34	32	42	37	29	39	34
	15.02.	30	38	34	31	45	38	24	36	30	32	40	36
-25ºC	15.12.	78	94	86	74	86	80	87	93	90	79	89	84
	15.01.	49	59	54	47	57	52	43	53	48	48	56	52
	15.02.	51	65	58	54	66	60	55	69	62	57	65	61
CABERNET FRANC													
-20ºC	15.12.	56	68	62	61	71	66	54	66	60	55	65	60
	15.01.	29	39	34	36	44	40	30	38	34	30	38	34
	15.02.	43	53	48	43	53	48	37	49	43	41	49	45
-25ºC	15.12.	75	85	80	82	90	86	78	86	82	72	82	77
	15.01.	53	63	58	45	57	51	50	58	54	48	58	53
	15.02.	46	54	50	48	60	54	54	64	59	47	57	57

*Source: Author's calculation.

The collected data were analysed using the three-way ANOVA to determine which factors affect changes in the percentage of frozen winter buds. The results reported in Table 2 show that all the sources of variability in the percentage of frozen winter buds in the observed varieties for all observed years have a statistically significant difference of 0.05. The exception is the variety as a source of variability in 2014/2015 (p=0.58) which leads us to the conclusion that there are no statistically significant differences in the number of frozen buds between the Merlot and Cabernet Franc varieties when it comes to other factors: temperature and date of measurement.

Such result led the authors to undertake a more detailed statistical analysis, which included a two-way ANOVA at individual levels of the third factor for the year 2014/2015 (Table 3). On that occasion, it was concluded that the difference between varieties as well as the difference

between the date of measurement and their interaction are statistically significant regardless of the freezing temperature. When the same analysis was performed for each variety, the same conclusion was reached: all key factors (temperature of freezing and date of measurement) as well as their interaction have a statistically significant influence on the differences in the percentage of frozen winter buds. No statistically significant difference was found for the variety at different measurement dates. On 15 December 2014, the result of measurement was p=0.588, on 15 January 2015, the result of measurement was p=0.708 and finally, the result on 15 February 2015 was p=0.900. These results show that in 2014/2015 regardless of the month in which the measurement was made, Merlot and Cabernet Franc varieties had a similar percentage of frozen winter buds. On 15 January 2015, the interaction was not statistically significant (p=0.708).

Table 2: Results of three-way ANOVA for the period 2011-2015

Year	2011/2012		2012/2013		2013/2014		2014/2015	
Source	F	Sig.	F	Sig.	F	Sig.	F	Sig.
Variety	7.19	0.01	15.17	0.00	6.88	0.01	1.32	0.58
Temperature	388.68	0.00	247.60	0.00	542.04	0.00	437.99	0.00
Date	242.56	0.00	249.93	0.00	264.44	0.00	250.89	0.00
Variety*Temperature	34.06	0.00	17.69	0.00	18.17	0.00	33.16	0.00
Variety*Date	1.47	0.63	3.23	0.05	1.32	0.28	1.19	0.91
Temperature*Date	13.91	0.00	5.85	0.01	20.67	0.00	7.94	0.00
Variety*Temperature*Date	9.43	0.00	2.84	0.07	19.06	0.00	10.30	0.00

*Source: Author's calculation.

Table 3: Results of two-way ANOVA for certain levels of the third factor 2014-2015

Variety (level of the factor)	**MERLOT**		**CABERNET FRANC**	
Source	F	Sig.	F	Sig.
Temperature	387.651	0.000	106.399	0.000
Date	133.087	0.000	119.044	0.000
Temperature*Date	11.342	0.000	7.231	0.003
Temperature (level of the factor)	**-20°C**		**-25°C**	
Source	F	Sig.	F	Sig.
Variety	21.892	0.000	12.417	0.002
Date	98.581	0.000	155.286	0.000
Variety*Date	5.473	0.001	4.968	0.016

Date (level of the factor)	**15 Dec 2014**		**15 Jan 2015**		**15 Feb 2015.**	
Source	F	Sig.	F	Sig.	F	Sig.
Variety	1.305	0.588	1.145	0.708	1.10	0.900
Temperature	190.840	0.000	139.219	0.000	108.936	0.000
Variety*Temperature	19.542	0.000	1.145	0.708	34.468	0.000

*Source: Author's calculation.

Table 2 shows that in addition to the variety, the interaction between the variety and the date of measurement was not statistically significant in all observed years: p=0.63 in 2011/2012, p=0.28 in 2013/2014, p=0.91 in 2014/2015, and in 2012/2013 the statistical significance had a border value of p=0.05, which means that the difference between the varieties is not statistically significant when it comes to the percentage of frozen winter buds in different winter months of measurement.

Finally, the LSD test was performed, as a continuation of the three-way ANOVA, which was supposed to show between which levels of individual factors there is statistically significant difference. However, the two factors, variety and temperature of freezing have only two levels each, so the LSD test was not done. The differences between the levels of these two factors are visible in Table 2. Only the third factor - the date of measurement has three levels, so it was necessary to test the differences between the individual levels (Table 4). This table clearly shows that in all years of observation there is statistically significant difference in the percentage of frozen winter buds when different dates of measurement are taken into account.

Table 4: Results of the LSD test for the factor *Date* for the period 2011-2015.

Date	Year			
	2011/2012	2012/2013	2013/2014	2014/2015
15.12.	70.25^{a}±0.915	72.00^{a}±0.926	70.25^{a}±0.880	68.00^{a}±0.822
15.01.	44.00^{b}±0.915	44.25^{b}±0.926	43.25^{b}±0.880	43.30^{b}±0.822
15.02.	47.50^{c}±0.915	50.00^{c}±0.926	48.50^{c}±0.880	48.50^{c}±0.822

*Source: Author's calculation.

Conclusions

In the research period 2011-2015, both Merlot and Cabernet Franc variety showed the highest resistance to low temperatures in January. The highest percentage of surviving winter buds was recorded during this month. Considering the full observation period, both varieties had the highest percentage of frozen winter buds in December, so it could be concluded that December is the most unfavourable month for both varieties.

The ANOVA showed that the percentage of frozen winter buds depends primarily on the variety, and then on the temperature and the date of the measurement. Throughout the observation period, the interaction between the variety and the date of measurement proved to be not statistically significant. The result is somewhat logical because both varieties had similar behaviour, in January they had the lowest and in December the highest percentage of frozen winter buds regardless of temperature.

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ANALYSIS OF GROWING AND TURNOVER OF TOBACCO IN REPUBLIC OF SERBIA

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Abstract

Raw tobacco production in the world has a long tradition and is widespread throughout the continents. Due to polymorphism as one of its most prominent traits, tobacco has adapted to all conditions under which it is grown. In addition to natural conditions, the most important of which are soil type and climate, raw tobacco production is also influenced by economic factors, such as the world market price of tobacco, government support and global anti-smoking policies. The aim of this paper is to present and analyze tobacco growing and turnover in Serbia, based on the collected data and information. The importance of the paper is in recognizing the structure and dynamics of production and marketing, as well as their causes, primarily because the production of this species brings significant economic benefits to the state, both through the realization of the products themselves, as well as through the creation of new jobs and income from taxes. The statistical data of the Statistical Office of Republic of Serbia, as well as the research results of the place and role of tobacco production in Republic of Serbia, served as the main source of data in the preparation of this paper. The data was analyzed using mathematical and statistical analysis, while relative dynamics indicators, baseline indices and average growth rates were used to demonstrate the status, changes and potential in tobacco production in Republic of Serbia.

Keywords: *tobacco, production, condition, market, selling.*

Introduction

In Serbia, tobacco production, processing and fabrication represent a unique industry. The tradition of tobacco cultivation in our country began at the end of the 19th century. Significant production was recorded after the First World War. During this period, our country was a well-known and world-renowned producer of small-leaved, oriental tobacco. Tobacco processing - production was dominated by oriental-type cigarette production. Efforts to obtain higher yields, reduce labor force participation and increase production efficiency have led to significant changes in the structure of tobacco production. In the last decades of the 20th century, production increase of large-leaved tobacco types has led to the complete displacement of oriental tobacco production in the Republic of Serbia.

Today, the tobacco market in Serbia is closely linked to the market for tobacco products, primarily cigarettes, with the world's largest multinational companies operating on this market: *Philip Morris International (PMI), Japan Tobacco International (JTI) and British American Tobacco (BAT).*

For the production of tobacco, it is useful to have a better knowledge of its technology, as well as the ability to achieve economically viable production. As more types of tobacco are produced, it is necessary to know the specificities in the production of each type individually (Hrgović, 2005).

The development and distribution of all branches of agricultural production, and therefore of tobacco, depends more or less on the climate. Serbia is located between approximately 42^{o} and 46^{o} north latitude. There are two major climatic regions in our country, the Pannonian and the mountainous. The Pannonian region is represented by the Pannonian-continental climate, slightly milder than the true continental climate that prevails in Eastern Europe, while the mountainous region is represented by three climatic types, temperate-continental, mountainous and parish. The presence and influence of different types of climate provides the opportunity to produce more types of tobacco.

Different types of soil are present in Serbia, which influences that tobacco production takes place on soil of different fertility, thus having an impact on yield and quality. Soil types in Serbia can be classified into two groups: soil in the plains and hilly regions, and soil in mountainous areas. The first group is dominated by chernozem, vertisol, cambisol and eutric cambisol, while the second group contains acid brown and parapodzolaceous soils, followed terra rossa, kambisol and rendzines (Đokic et al., 2015). Different types of climate and soil require respect for the needs of tobacco in terms of conditions for its successful cultivation, which is often neglected in practice. For this reason, we face the fact that there is a lack of classification by regions of tobacco types and varieties, which adversely affects the yield and quality of the raw material.

In Serbia, in recent years, there has been a slight increase in transplanted and cultivated areas, an oscillation of average yields, and a decrease in the number of holdings engaged in this production. The yield variations per hectare are the result of economic exhaustion and stem from the inability to implement all the necessary cultural practices and tobacco protection measures. Support for tobacco growers in Serbia was discontinued in 2011, so today it is the biggest drawback in the production of this crop in our country.

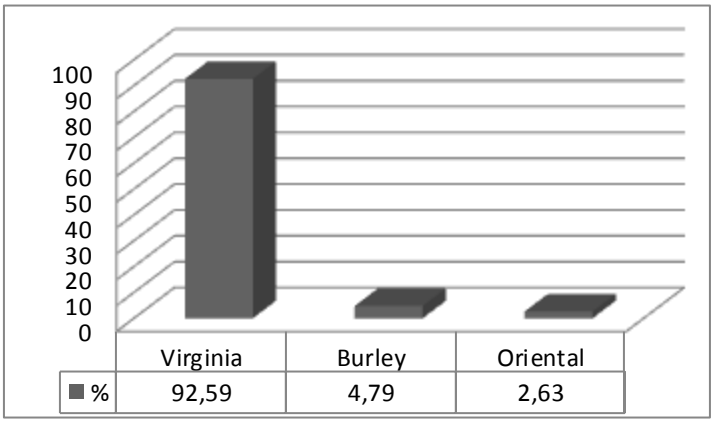
The economic importance of tobacco

Tobacco in the leaf and its products represent significant products in the world economy of the world, and occupy a high place in international trade in goods. In the economy of some countries, it occupies a very important place, for example, tobacco in US is just behind wheat, cotton and maize, with about 8% share (www.fao.org). The national economies of Greece, Bulgaria, Turkey and several other countries are highly dependent on the volume of tobacco production and export. The importance of tobacco production in Serbia is reflected in a significant share in the formation of national income, income generated through taxes (which is one of the important revenues for the country's budget), etc. Tobacco production in the leaf requires considerable labor force participation, from production in the field to the production of finished products, which makes it important for tobacco, both in the formation of national income and in improving the country's balance of payments (Đokić et al., 2015).

Structure of tobacco production in the Republic of Serbia

Serbia has a very long tradition of tobacco production since the second half of the 19th century, when the first small factories for the purchase and primary processing of tobacco were established. Almost all domestic tobacco production takes place on individual holdings, so this branch has a specific economic and social importance. Tobacco cultivation has traditionally been present in underdeveloped regions, especially in the south of the country. Since the 1970s, plantation cultivation of high-yielding, broad-leaved and high-quality tobacco varieties in lowland areas has been increasingly present in Serbia, but significant production is still taking place on small plots, with specific microclimate, soil quality, low level of technical and technological equipment and applications of modern cultural practices. In terms of economic importance, it is enough to mention that about 15.000 families in Serbia live and depend on tobacco production. Taking into account the seasonal labor force,

processing industry employees, service workers, manufacturers and suppliers of required materials, this number would increase many times over (Radojičić, 2011).
According to data of the Ministry of Agriculture, tobacco production in Serbia ranges from 6.842 to 9.341 tons on areas between 4.691 and 5.256 hectares and average yields of 1.4 to 1.9 t ha^{-1}. In 2015, Virginia is the most represented in the production structure with about 93%, Burley with about 5%, while Oriental tobacco occupies about 2.5% of the area (Chart 1). Since 2016, Oriental tobacco is no longer produced in Republic of Serbia and production of Burley type tobacco has been declining over the years.



Graph 1. Structure of tobacco production in Republic of Serbia in 2015.
Source: http://www.duvan.gov.rs/

Tobacco market in Serbia

Due to the strong interdependence of the tobacco market and the tobacco products market, it is best to observe it as a single market. In this situation, key entities can be divided into three groups: producers, consumers and the state. In the production of tobacco and tobacco products, there are tobacco manufacturers, tobacco processors and manufacturers of tobacco products. In addition to these three entities, there are also companies involved in the production of tobacco inputs, transport, wholesale and retail trade.
Tobacco production in Serbia is a good example of the functioning of the agro-industrial complex (Tomin and Đorović, 1989), which consists of three significant parts: the pre-farm, farmer and post-farm sectors. The pre-farm sector includes input manufacturing companies, and in the case of tobacco production, this sector includes enterprises that produce tobacco drying driers, specialized planters or chemical agents for tobacco production. The farmer sector is represented by tobacco producers, while the post-farmer sector includes the tobacco industry, tobacco processors and manufacturers of tobacco products, wholesalers and retailers, as well as transport companies (Veljković, 2006).
Tobacco producers in Serbia are exclusively farms that produce tobacco in cooperation with tobacco processors. The number of agricultural holdings producing tobacco decreased from 2006 to 2012 from 3,922 to 1,765. However, the average size of the holding increased from 1.6 ha to 3.56 ha. The number of tobacco producers is greatly influenced by changes in the agrarian policy of the country, where the abolition of subsidies for tobacco production influences giving up this production by some producers (Đokić et al., 2015).
Tobacco processors are business entities that carry out the processing (one type of fermentation) and packaging of tobacco leaf or strips. According to the Tobacco Law, tobacco processors are obliged to have contracts or pre-contracts with tobacco producers, and because of the specific business relationships between these two entities, they are defined as organizers of production on the farm. In addition to tobacco seeds or seedlings, production

organizers also provide advances in the form of reproductive material, protective equipment, greenhouses, dryers, etc., this way giving credit to their subcontractors (who, depending on the size of the investment, repay advances at the end of the season across tobacco, while for major investments such as dryers and greenhouses, producers make repayments over the next few years). The processors also organize training for producers in the form of seminars and experimental field visits to give them the opportunity to practically see the benefits of new technologies available.

Tobacco processors, who are also organizers of production in Serbia, are the *Tobacco Industry Čoka, Tobacco Ljubovija*, and *JTI Senta*, which also manufactures tobacco products. In addition to the listed companies, there are tobacco companies in the tobacco market that are only production organizers, but not tobacco processors. The most important of these companies are *Monus* from Belgrade, *COPEX* from Novi Sad, and until 2017, *Alliance One Tobacco* from Belgrade, which organized tobacco production and purchased tobacco for *PMI*. They process tobacco by paying for tobacco processing services to other companies.

Manufacturers of tobacco products are economic operators producing the final tobacco products, primarily cigarettes. The largest multinational companies such as *PMI* (formerly DIN), *BAT* (formerly DIV) and *JTI* (formerly the Senta Tobacco Industry) operate in the territory of Serbia. In addition to these three companies, the production of tobacco products is also carried out by the company *Monus* from Zemun (Đokić et al., 2015).

In the market of tobacco and tobacco products of Serbia, the state has a multiple role. Through its agrarian policy measures, it influences tobacco production by giving different types of subsidies to tobacco producers. Such support also affects the business of other entities, above all tobacco processors. Through excise duties, the state also affects the price of cigarettes, thereby generating revenue and potentially reducing consumption, leading to reduced demand for raw tobacco. It also regulates the market through appropriate legislation. Through its agrarian policy, the state influenced the income of tobacco producers in various ways, so that from 2001 to 2011, tobacco production was subsidized through premiums per kilogram of manufactured tobacco or through premiums per hectare of planted tobacco. After 2011, the state no longer encourages tobacco production through special measures.

Consumers buy and consume the final products of the tobacco industry, but also indirectly generate demand in the tobacco market (the increase in consumption of tobacco products affects the demand for tobacco). According to the latest data, Serbia holds the first place in the world in per capita consumption of cigarettes, so as many as 2,861 per capita cigarettes are consumed annually (www.tobaccoatlas.org). If we omit the fact that this information is extremely disturbing on the health side, on the economic side it speaks to the high demand for tobacco products and the need for strong domestic production to make the foreign trade balance as favorable as possible.

Table 1. Tobacco production and average annual purchase prices of unprocessed tobacco from 2010 to 2016

Year	Republic of Serbia			
	Harvested area (ha)	Total yield (t)	Yield ($t ha^{-1}$)	Redemption price (RSD)
2010	4,691	8,402	1.8	193.52
2011	5,139	8,192	1.6	191.55
2012	5,050	6,842	1.4	213.52
2013	4,939	7,977	1.6	244.24
2014	4,899	9,341	1.9	233.68
2015	5,012	8,776	1.8	221.71
2016	5,256	7,810	1.5	246.42

*without data for AP Kosovo and Metohia

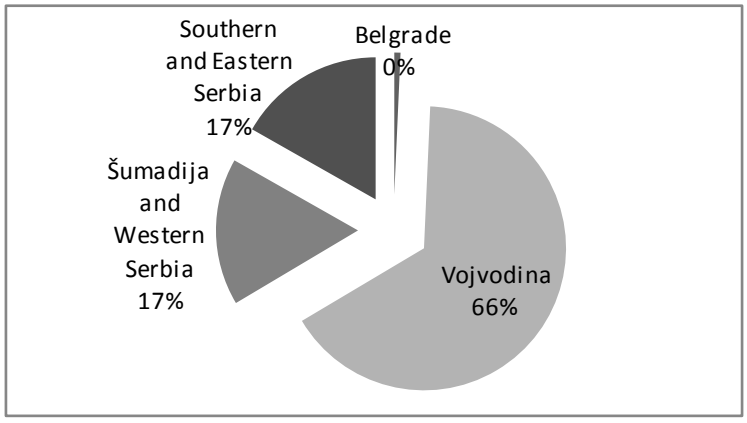
Source: Statistical calendar of Republic of Serbia (2010-2016)

In the structure of field crop production in Serbia, tobacco has taken a marginal position in the past ten years. The largest areas under tobacco in Serbia in the analyzed period (2010-2016) were recorded in 2016 and amounted to 5,139 ha, while the highest production volume of 9,341 tons was recorded in 2014. In the observed period, the total cultivated area under tobacco fluctuated, and also the yield achieved by years recorded many deviations. Yield per hectare was the highest in 2009 (1.9 t ha^{-1}), while the lowest was noted in 2012 (Table 1).
In this period, total yields did not vary much, variations were most likely present due to the high dependence of tobacco production on weather conditions. The average purchase price of a kilogram of tobacco increased significantly in the observed period, with the highest level being achieved in 2013 and 2016 (around 245 dinars per kilogram of tobacco). In addition to the supply and demand ratio in the domestic and world markets, one of the factors that certainly has a great influence on the price movement is the state policy towards this production. The total area under tobacco in Republic of Serbia during the observed period recorded a slight increase, although since 2011 there are no longer subsidies from the state that are intended to encourage this production. The highest harvested areas and highest yield per hectare were recorded in 2016 in Vojvodina. Significant areas under this crop were also recorded in Southern and Eastern Serbia (1,081 ha), as well as in Šumadija and Western Serbia (871 ha), with slightly lower yields per unit of capacity. The smallest area under tobacco in 2016 was recorded in Belgrade (Table 2, Graph 2).

Table 2. Tobacco production by regions in Republic of Serbia in 2016

Region	Tobacco		
	Harvested area (ha)	Total yield (t)	Yield (t ha^{-1})
REPUBLIC OF SERBIA*	5.256	7.810	1,5
Belgrade	39	55	1,4
Vojvodina	3.265	5.136	1,6
Šumadija and Western Serbia	871	1.308	1,5
Southern and Eastern Serbia	1.081	1.311	1,2

*without data for AP Kosovo and Metohia
Source: Statistical Office of the Republic of Serbia, Belgrade



Graph 2. The structure of tobacco production in the regions of Republic of Serbia in 2016
Source: Statistical Office of the Republic of Serbia, Belgrade

Turnover of tobacco

The foreign trade balance represents the value difference between export and import of the country in international trade. In the period from 2010 to 2016, Serbia's foreign trade balance

was negative in the first years, and from 2014 it was positive (Table 3). A positive balance in recent years has emerged from a significantly higher increase in export of tobacco products, compared to a slightly smaller increase in import.

Table 3. External trade of tobacco products from 2010 to 2016 (in millions of Euros)

Year	Republic of Serbia		
	Export	Import	Balance
2010.	34,9	93,1	-58,2
2011.	31,7	83,4	-51,7
2012.	30,9	82,8	-51,9
2013.	73,8	118,6	-44,8
2014.	132,6	123,2	9,4
2015.	222,9	192,5	30,4
2016.	358,8	195,1	163,7

Source: Statistical calendar of Republic of Serbia (2010-2016)

Production of tobacco without subsidies

The tobacco production market stands out from others. It is very important for tobacco production to secure a placement, but also to achieve the best purchase price for tobacco, which can fluctuate a lot. The price of around 246 dinars per kilogram in 2016 for the first class of tobacco represents the value at which producers earn a minimum profit when producing this crop.

In all countries where tobacco is grown, the state provides premiums to encourage the activities of the agricultural population (Hrgović, 2005). In some countries, the state also mandates a minimum purchase price below which the tobacco industry must not go when buying tobacco from producers and the motives for tobacco dying are usually to ensure a high and stable price, support small farms and increase the competitiveness of domestic tobacco. Serbia also used tobacco remediation measures as part of its policy to support agricultural competitiveness and rural development, but is no longer using it today (Matkovski and Đokić, 2014).

This is also one of the major problems that accompanies this production in our country. The investments in this type of production are extremely large, it requires a lot of labor, and the costs, especially of tobacco drying, are quite high. Fertilization costs range from 9 to 12% in the structure of total production costs. The share of protective equipment costs in the structure of total costs ranges up to 7.40%. However, of all cost items, energy costs have the largest share in the structure. They account for 26 to 28% of total costs (Radojičić, 2011). Tobacco producers, while subsidies existed, organized production more easily than they do today. This is primarily reflected in the inability to achieve a more secure and higher profit today, which would allow further investment in production (e.g. investments in necessary machinery, purchase of dryers, etc.).

Since there are no subsidies from the state, growing tobacco requires great costs, especially for small producers. On the other hand, by working with large companies that provide seed, material and secure placement, much better results can be achieved, even more cost-effective than some standard field crop species (Strategy of Agriculture and Rural Development of Republic of Serbia for the period 2014-2024).

Conclusion

There are five key entities in the tobacco market in Serbia: tobacco producers, tobacco processors, producers of tobacco products, the state and consumers. Republic of Serbia has a multifaceted role in the market of tobacco and tobacco products, which is manifested primarily through agrarian policy measures, thereby affecting tobacco production. Also, by

laws and regulations through the competent ministries, the state regulates both the conditions of production, processing and fabrication, as well as the excise policy, through which it replenishes its budget.

In the observed period (from 2010 to 2016), the yields on tobacco production had varied, most probably under the influence of weather conditions for each year. The largest cultivated areas in 2016 were recorded in Vojvodina (3,265 ha), where the highest yield per hectare (1.6 t ha^{-1}) was also recorded. Significant areas under this crop were recorded in the Southern and Eastern Serbia (1,081 ha), as well as in Šumadija and Western Serbia (871 ha). In the analyzed period, the average purchase price of unprocessed tobacco kilograms increased significantly and the highest level was achieved in 2016 with 246 dinars for the first class. Considering that the subsidization of tobacco production by the state has been abolished since 2011, it can be concluded that the organizers of production have tried to stimulate producers with higher purchase price of tobacco.

The foreign trade balance of tobacco products has a positive tendency, so a surplus has been recorded in recent years, compared to the deficit from previous years.

Acknowledgments

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http://www.fao.org/

http://www.duvan.gov.rs/

APPLICATION OF STUTOX II. WITHIN AN EXTRAORDINARY SITUATION OF THE COMMON VOLE OVERPOPULATION IN THE AGRICULTURAL LANDSCAPE AND ITS LEGAL ASPECTS

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Abstract

At this time we face the exceptional situation of overpopulation of common vole in the Czech republic due to which significant damage arises in agricultural areas. Increased common vole population from 2016 to 2019 could be observed not only in the Czech Republic (2,4 times more) but also in Germany (2 times more), in Spain (7,4 /3,3 times more), in the Netherlands (5,9 times more) and in Hungary (2 times more). This article focus on the legal instruments by which farmers can defend their plant production against pests, in particular on the use of the plant protection product Stutox II and the regulation of these instruments with a simultaneous impact on biodiversity in the local (affected) areas. Stutox II. can be applied basically in two ways – directly into the rodent burrow or by spreading on the surface of the affected field. Farmers prefer the latter variant during a calamity situation – however, it is a threat to biodiversity, especially for wildlife. Unfortunately, the most endangered group are the natural enemies of the common vole, who often eat poison through their dead bodies. This exacerbates the problem of vole overgrowth. Other endangered animals eat the poison directly because of its nutritional content and the fact that it was applied to the field by spreading on its surface. Therefore we will analyze and critically evaluate relevant legal instruments, decipher collision between biodiversity and protection of plant and suggest some alternative solutions, including also farming method, which could help to avoid another common vole calamity and which would be even a beneficial for the environment.

Keywords: *Common vole, Overgrowth, Widespread application of poison.*

Introduction

During the last three years, it has been possible to observe overpopulation of the agricultural landscape by the common vole. It is a typical inhabitant of these areas, but its populational growth creates immense production losses, and the farmers must overcome them (Ekolist.cz, 2019). Voles, however, do not create risk only for the farmers but also act like dangerous disease vectors (Tkadlec, 2019). In the present, widely discussed is preparation Stutox II, which can be, if the legal conditions are met, used by farmers directly into the burrows or indirectly by spreading on the fields. The goal of this paper is to outline these problems from the legal point of view because the legislation of this problem is not intelligible nor straightforward. Firstly, it is necessary to realize that usage of the preparation Stutox II creates a conflict of two interests. There is a business right of the farmers and prevention of spreading of the diseases, but Stutox II can have a harmful or deadly effect even on the non-target organisms (Mach, 2019). Therefore, the legislators currently make efforts to create a compromise solution, which would take both sides into account. The paper will describe these and conclude with possible alternative solutions or means of prevention of further vole overpopulation. The information corresponds with data to 10th March 2020. The authors did empiric research, where they focused on the real status of dealt problematics. They used one of the general scientific methods – the analysis when they focused on the historical consequences of the given situation following with introduction and examination of the

legislation related to the issue. Based on this research, the authors presented the potential alternative solutions

Historical evolution of the common vole population density in the Czech Republic

The Czech Republic currently deals with the overpopulation of the typical example of rodents living in the farmland – the common vole (*Microtus arvalis*). Although its momentarily paid special attention, from the historical point of view it is not a completely exceptional situation. The so-called fluctuation phenomenon is typical for this rodent, it is "*irregular change in the population size, and if these changes start to occur with specific regularity, they become periodical, we call them shortened population cycles*" (Martincová, 2012). The oldest preserved record to prove this phenomenon on the territory of the Czech Republic comes from 1925. Farský (forester, scholarly writer) not only describes the given situation in detail but also tries to argue over possibilities of stopping the phenomenon and means of prevention (Tkadlec, 2019). Although there were countless overpopulations of the common vole locally in the past, the current status is after all different. The research from the Mendel University Brno states that the ongoing damage done by the rodent is more substantial than with any of the previous overpopulations. This is the main reason to create such measures, that would prevent additional damage in the future. However, there is by no means effort of total extermination of the species. Such a step could be critical interference of the food chain, where the vole plays an irreplaceable role. Therefore the goal is only to lower the numbers of the currently oversized population (Ekolist, 2019).

Population density of the common vole in the Czech Republic started to rise in 2018. Because there were no adverse effects on the species, such as cold winter, long rain period or fatal disease, the population growth in 2019 continued without significant problems (Mach, 2020). The statistical data show that by comparison of the field area with burrow exits, it is highly probable that the population trend will also continue this year. However, the question of whether the situation of 2019 will repeat will be decided by the evolution of vole population density in June. From a geographic point of view, more voles can be observed in the historical region of Bohemia rather than Moravia. This is attributed to different climatic conditions (Tkadlec, 2019). Given the average harmfulness, the worst situation is in the Vysočina region, and the best is in the Liberecký and Karlovarský region (The Ministry of Agriculture of the Czech Republic, 2020). The current occurrence of the common vole can also be found on the Czech Phytopathological portal in the occurrence map section (The Ministry of Agriculture of the Czech Republic, 2020a).

The study by Dr Jens Jacob from Institute of Julius Kühn (German research institute of crops) shows that the population growth of the common vole also affects other European countries. Increase of population between years 2016 and 2019 had been observed not only in the Czech Republic (2.4×) but also in Germany (2×), Spain (7.4×/3.3×), Netherlands (5.9×) and Hungary (2×). France reports a decrease during the period. Although the other European countries such as Austria, Belgium or Switzerland were not included in the study, by different (non-quantified) sources, these countries also observed an increase of the vole populations (Bouma, 2020).

Stutox II and his application to decrease the population of overpopulated voles

Farmers use different means of protection in the control of overpopulated voles – mechanically, chemically, or by scattering of farmland and landscape elements. The paper focuses on the application of rodenticide Stutox II used for the control of harmful organisms (Prokop, 2017). The name of the given product suggests the existence of a predecessor. Stutox I was refrained from usage on 23rd August 2018, mainly because it was causing too drastic mean of death to the rodents (preparation register).

Stutox II is a plant protection product registered in the Plant Protection Product Registry of the Central Institute for Supervising and Testing in Agriculture (hereinafter CISTA) under

registration number 5114-0. It contains 2.5 % of the active substance – zinc phosphide. This preparation can be used based on a decision from 15th November 2018 until 30th April 2025 (Plant Protection Product Registry). The zinc phosphide is a grey-black powder, which decomposes under the influence of a gastric environment producing poisonous gas hydrogen phosphide (PH_3), which stops mitochondrial processes. Death occurs depending on the dosage within 3 to 5 hours (Tkadlec, 2019). Stutox II is used in the form of granules, which contain active substance compressed together with alfalfa, to be more attractive for the pest. Vole can be killed by ingestion at least two pellets. Poison later expires from the body leaving harmless substances, which naturally occur in the soil, so it does not damage landscape or any other environmental component (CISTA, 2019). To be specific, the zinc phosphide decomposes into a small amount of the phosphine gas, which escapes into the atmosphere and other non-toxic substances including zinc, which is a natural compound of the soil. (CISTA, 2020).

Application of Stutox II directly into burrows

The current legal order allows two types of usage of preparation Stutox II – placing directly into burrows and indirectly spreading on fields. Roots of legislation for the usage of the active substance in agriculture are in Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21st October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC, whose content focused on the usage of the active substance is carried out in Act No. 126/2004 Sb., o rostlinolékařské péči (Phytosanitary Care Act – hereinafter PCA). The burrow application was allowed by § 37a par. 1 PCA following Article 53 of the Regulation by Central Institute for Supervising and Testing in Agriculture about the allowance of the plant protection product for limited and controlled usage from summer 2019. Subsequently, another was published in February and March 2020, allowing the application of Stutox II into burrows until June. However, the insertion of pellets into every burrow individually is for the farmers physically impossible.

Spreading Stutox II and influence on biodiversity

If we talk about the impact on biodiversity in connection with Stutox, this does not mean the targeted control of voles, whose population has just overgrown. The result of its widespread application on the other parts of the environment, especially on wild animals, is essential. Killing effects of poison do not only affect the target group but also causes death to others, often protected and rare animals. Ministry of the Environment (hereinafter MoE) states that the widespread application of poisons subsequently reduces the population of vole predators, worsening the situation for the upcoming years. This even brings additional costs for the MoE in need of various subsidiaries and rescue programs to increase predator numbers. (MoE, 2020).

Undesirable poisoning endangers other animals that occur in the locality in two ways. Primary poisoning occurs when the poisonous granule is consumed directly by a non-target group of animals for its high forage content – most frequently hares, pheasants and others. Secondary poisoning consists of the consumption of already poisoned animals, especially a vole, which is even more harmful, as it leads to the loss of an already insufficient number of natural predators, especially predator birds and storks. For these reasons, the widespread surface application of the poison is very carefully considered, and the CISTA with the Ministry of Agriculture (hereinafter MoA) is looking for the most suitable ways to solve this complicated situation. In August 2019, the CISTA issued regulation, which established an exemption from the PCA and allowed the widespread application of a poison in large parts of the country. However, a wave of opposition has been raised by conservationists and the MoE, which has led to the suspension of the regulation. Although the regulation was only effective for a few days, almost 80 dead hares, several pheasants and a stork were found in Moravia, where tests

resulted in the presence of poison. Therefore, the poison was again applied only to the burrows (MoA, 2020b).

In connection with a surface application, the MoE began to appeal for the protection of specially protected species according to Section 56 of Act No. 114/1992 Sb., o ochraně přírody a krajiny (Nature and Landscape Protection Act – hereinafter NLPA), who may occur in the concerned area. Regional authorities are currently overwhelmed by these exemptions (the most affected region is the South Moravian Region with almost 130 requests as of 29th February 2020). Interestingly, none of the requests has been complied with yet, and the exemption has not been granted, due to the prevailing interest in nature protection (Mach, 2020). At the beginning of March, however, the CISTA issued three more regulations concerning the application of poisons in the intentions of the current calamity situation. A third of them correspond to the issue of widespread surface application of poison, which allows the spreading of Stutox II on the surface using a spreader on land with calamitous overpopulation in the amount of a maximum of 10 kg/ha. Calamity overpopulation is defined as *"such an intensity of its occurrence on a specific plot when five times its current threshold of harmfulness for the crop on the given plot is reached or exceeded."*(Regulation of CISTA, 2020). The fact that there is a calamity overpayment will be examined on the spot by inspectors from the CISTA. Following their findings, they issue a protocol/report on the overpopulation status, which conditions the subsequent authorization of the poison application. The possibility that inspectors could also order emergency phytosanitary measures, which take the form of decisions and can be appealed against, was also discussed. These measures were initially created for other purposes (rather quarantine), but it is not possible to exclude their use in this situation (Mach, 2020). Thus, although the poison can be applied to agricultural land by spreading under certain conditions, it is subject to a myriad of protective conditions, including, for example, the need to remove vole carcasses from the treated land at least once a day for at least 48 hours after application. The corpses of rodents must then be buried in a suitable place to a depth of at least 80 cm, using disinfectants. The appropriate location is determined from the protection of human, animal, and environmental health (Regulation of CISTA, 2020).

The exception mentioned above (not only) from the prohibition of capture, hold in captivity, disturbance, injury or killing of specially protected animals, enshrined in the provision of § 56 NLPA, can be considered worthy of consideration. According to it, an exception to those prohibitions is permitted in cases where another public interest outweighs the interest of nature conservation, and the authority concerned is a nature conservation authority. In practice, the nature protection authority dealing with the request for this exemption views the occurrence of specially protected animal species in a specific locality on two levels. The first of them can include specially protected animals that are tied to the site and can be found in the occurrence database, which is the result of monitoring provided by the Nature and Landscape Protection Agency of the Czech Republic. Suppose based on this finding the nature protection authority concludes that the permanent occurrence of specially protected animals is not found in the given locality. In that case, it is necessary to take into account the second level of their possible presence – this includes especially protected animals that may occur in the given area. Unpredictably, primarily due to migration. These are mainly bird species. For these reasons, it is necessary in all cases to obtain an exception for the surface application of poison according to § 56 NLPA. In some cases, there is a risk of primary poisoning, but in the vast majority of cases, secondary poisoning is more likely to occur. The arguments of the regional authorities for not granting an exemption are thus based mainly on the group of migrating specially protected animals. The question remains whether this attitude is correct or whether it is only a way to prevent the surface application of Stutox II. We may also be led to think about why in this case only specially protected animal species are at the centre of biodiversity

conservation and no longer, for example, wild birds, for the threat of which an exemption under § 5b ZOPK is also needed. The law also prohibits their killing, but with the difference that there is an *intentional* killing prohibited. This fact could be a guide to the answer to why regional authorities currently require an exemption only for specially protected animal species?

The mutual comparison of public interests also plays a crucial role in the question of allowing the application of poison to the mentioned extent. Is there a greater interest in nature conservation or in crops that are essential for society? Let us now focus on the interest of farmers (hence society) in the bountiful harvest. Undoubtedly, agricultural entrepreneurs have the primary goal of generating profit (Židek, 2015). However, there are two types of agrarian entrepreneurs - the landowners themselves and the lessors. Lessor's relationship to the land is based on a lease agreement by which he or she (i.e. in this case the owner of the land) undertakes to leave the thing (i.e. the land) to the lessee for temporary use and enjoyment. The lessee undertakes to pay the lessor a rent or a proportionate part of the rental income by the § 2332 of the Act No. 89/2012 Sb., občanský zákoník (Civil Code). Furthermore, the statutory provision of the Civil Code stipulates the duty of a tenant to take care of the matter with the diligence of a professional manager. This vague term to which an ad hoc court gives a specific meaning already carries almost the care of the owner in the context of a linguistic interpretation. It cannot be assumed that every owner takes care of his thing with reason, responsibility and relation to it, but in general, the tenant should by law treat the leased thing in the same way as its owner. However, these people are in different positions, especially as regards the relationship to land, it can be assumed that despite all due care, there will be a difference between the tenant and the owner, for example in choosing the methods farmers approach a particular issue. Lessees will choose mostly short-term and cheaper tools or methods that will currently help increase profits. On the contrary, the owner will approach the land with a longer-term plan and will use tools that support the land and the entire ecosystem there, even at the cost of a longer-term process with less current profitability. From the solved topic, it can be assumed the surface application of Stutox II will rather be the choice of tenants, and in the case of owners, it will be the use of alternative tools, which will be outlined in the next chapter.

The alternative solution to the usage of preparation Stutox II

From all facts mentioned above, we could deduce steps which could help our country to avoid a similar overpopulation of the common vole which excludes the usage of Stutox II in the coming years. The results of this article we could find in upcoming alternative solutions which we present here.

Many experts identically recommend deep ploughing, which should be done after harvest, ideally at the end of summer or in autumn. This season by itself is unfavourable for voles because they lose their constant supply of food. When there is concurrent ploughing in range 20 to 30 cm (the burrows are about 10 to 20 cm below surface), it has almost destructive consequences. It is observed that the population in the case of deep ploughing lowers by 90 % on average. Sophisticated and much more effective solution is the heterogeneous landscape arrangement. It is characterized mostly by the diversity of landscape elements including for example draws, division of area to smaller cultivated fields, the richer structure of the crops, forested areas and areas with the character of a meadow. However, even this arrangement is not 100 % effective against voles; its positives are mostly in fact, that developmental stages of the rodent are not synchronous, so the overpopulation of the vole cannot reach such level as in the case of the non-heterogenous landscape. This method was mainly successful in France (Tkadlec, 2019). This type of landscape is also home for different predators of the common vole (Czech News Agency, 2019). Another solution is to let nature to deal with the situation by itself. Following statements from the beginning, common vole overpopulation is not a

unique case as it repeats itself in specific intervals, meaning raising the population always reaches such peak it inevitably collapses. Thus, the solution lies in the farmers not fighting with voles at all. The issue is compensation for farmers. The final solution is to continue using Stutox II, but it is necessary for the MoE with the MoA to resolve and state real conditions for doing so. The burrow application is defined correctly, but spreading of the rodenticide is, due to exception in § 56, currently impossible.

Conclusions

The paper presented the exceptional overpopulation of the common vole in the Czech Republic and the legal basis for the use of rodenticide Stutox II. Despite all efforts to produce an environmentally friendly product, it is not possible to rule out its negative impact on non-target organisms. As mentioned, the primary emphasis should be on the original layout of the landscape and the way of farming, to prevent these and similar calamities. Properly functioning ecosystems can deal with many hardships on their own without the need for human intervention. However, disrupting the natural harmony, extreme situations will begin to occur that nature can no longer solve by itself. Then comes a more radical solution, most often using chemical products (such as Stutox II). So if something does not change in our access to the agricultural landscape, we will have to continue to face similar calamities and decide between two evils. The reaction from the authorities was relatively rapid, due to conflicting views and frequent changes in legal regulation; it was also very inconsistent, resulting in low legal certainty for the entities concerned. Farmers were unable to orientate in the situation; applying to regional authorities for exemptions without even knowing what for. However, the biggest paradox is that although the widespread application is allowed under specified conditions, in practice, the exceptions needed to do so are hardly issued. For other similar situations, it would be appropriate to properly discuss the situation with experts from all areas concerned and, on that basis, to issue a comprehensive legal act providing a useful tool in the fight against overpopulated rodents, which would be available to farmers. However, the best solution would be the broader concept of agricultural change and treatment of the agricultural landscape outlined above, which would help to prevent these extraordinary situations as much as possible.

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CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF FRUITS OF THREE PLUM CULTIVARS GRAFTED ON FOUR ROOTSTOCKS

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Abstract

Chemical and antioxidant properties of fruits of three plum cultivars ('Čacanska Rana', 'Čacanska Lepotica' and 'Čacanska Najbolja') grafted on four rootstocks (Myrobalan, 'Pixy', 'Fereley' and 'St. Julien A') were studied in the region of Belgrade (Serbia). Chemical composition of plum fruits was found to be more cultivar- then rootstock-dependent. The average values for soluble solids, total sugars, inverted sugars, sucrose, and total acids content in cultivar/rootstocks combinations ranged from 11.7 to 14.2%, from 9.5 to 11.5%, from 6.6 to 7.6%, from 2.7 to 3.9%, and from 0.71 to1.11%, respectively. The contents of soluble solids and total sugars were the highest in fruits of 'Čacanska Najbolja', while the lowest contents were found in fruits of 'Čacanska Rana' cultivar. Total phenolic content (TPC) in the skin and flesh of plums was in the range from 4.44 to 15.93 mg GAE g^{-1}, and from 0.38 to 0.86 mg GAE g^{-1} respectively. TPC in the skin was 6–20 times higher than in the flesh of the same cultivar/rootstock combinations. Differences in TPC among rootstocks were not statistically significant, but significant differences among cultivars were found. Radical-scavenging activity (RSA) in the skin and flesh ranged from 39.08 to 78.49 μmol TE g^{-1}, and from 10.40 to 16.97 μmol TE g^{-1} respectively. Significant differences in RSA were found among cultivars and cultivar/rootstock combinations, whereas differences between rootstocks were not significant. The highest RSA was found in fruits of 'Čacanska Najbolja' cultivar.

Keywords: *Prunus domestica, cultivar/rootstock combinations, total phenolic content, radical-scavenging activity.*

Introduction

One of the most important commodities, consumed worldwide, owing to its degree of acceptance by consumers is plum fruit. Plums and their products have many beneficial effects on human health: improving bone health, antioxidant and anti-inflammatory activity, improving memory, reducing the risk of cardiovascular diseases, possess laxative effects, anti-allergy, and antimicrobial activity (Igwe and Charlton, 2016). This fruits are considered as a functional food because of the high content of bioactive compounds such as dietary fiber, sorbitol, phenolic compounds, and minerals (Stacewicz-Sapuntzakis et al., 2001). According Bohačenko et al. (2010), in addition to economic and physical parameters, such as yield, weight of fruit, shape, color of skin, stone mass, sensitivity of the fruit to biotic and abiotic factors, in the commercial use of the fruit, the chemical parameters also have an important role.

Thanks to the high sugar content, fruits of fresh plums, and especially dried plums (prunes), are an excellent source of energy. Although the main sugars in the plum fruit are monosaccharides, they do not affect the rapid increase in blood sugar levels due to the high

content of fibers and sorbitol (Eskin and Snait, 2006). Dominant sugar in plum fruit is glucose, followed by sorbitol, sucrose and fructose (Stacewicz-Sapuntzakis et al., 2001).
Phenolic compounds are biologically active substances having antioxidant properties and positive effects on human health (Walkowiak-Tomczak, 2008). The main phenolic compounds in the plum fruits are caffeic acid derivatives: neochlorogenic acid (3-*O*-caffeoylquinic acid), and chlorogenic acid (5-*O*-caffeoylquinic acid) (Stacewicz-Sapuntzakis et al., 2001). The skin contains about five times more phenolic substances than the flesh (Stacewicz-Sapuntzakis et al., 2001).
Antioxidant activity of plum fruits is higher in comparison to other pome and stone fruits, with the exception of sour cherries. In relation to the apple it is two to four times higher (Wang et al., 1996; Halvorsen et al., 2002; Kim et al., 2003; Cho et al., 2007). The antioxidant activity values are significantly higher in the skin than in the flesh of the fruits, which can be explained by the higher content of total phenolic compounds and anthocyanins (Stacewicz-Sapuntzakis et al., 2001).
Rootstocks can affect not only the vegetative growth and yield, but also the fruit quality. Although fruit quality is mostly cultivar-associated trait, the significant effects of rootstocks on fruit quality in different fruit crops have been reported (Usenik et al., 2010; Orazem et al., 2011; Bartolini et al., 2014; Reig et al., 2016; Font i Forcada et al., 2019; Iglesias et al., 2019; Milošević et al., 2020).
The influence of rootstocks on plum fruit quality attributes have been poorly studied, especially in the European plum. Most of the work has been related to their influence on the content of soluble solids and titratable acids in the fruit (Daza et al., 2008; Rato et al., 2008). The effects of the rootstocks on the composition of sugars and phenolic compounds in plum fruits have not thus far been investigated in detail. Therefore, the aim of this study was to determine the influence of three cultivars and four rootstocks on plum fruit chemical composition and antioxidant activity.

Material and methods

The fruits were taken from the plum orchard at the Experimental Station 'Radmilovac' of the Faculty of Agriculture in Belgrade (Serbia). Four rootstocks were included: one seedling rootstock (Myrobalan) and three clonal rootstocks ('Pixy', 'Fereley' and 'St. Julien A'). Three table plums cultivars ('Cacanska Rana', 'Cacanska Lepotica' and 'Cacanska Najbolja') were grafted on each rootstock. Every combination cultivar/rootstock was represented by six trees. The orchard was planted in 2010. Planting distance was 4 m between rows, and in the row different distances were applied depending on the rootstock vigor: 2.3 m for Myrobalan seedling, 2.0 m for 'Fereley' and 'St. Julien A' and 1.7 m for 'Pixy'. Training system was the Spindle. Standard cultural practices were applied, including drip irrigation. From every combination cultivar/rootstock, 50 fruits were picked at commercial maturity and used for chemical analyses. Chemical composition of fruits of three plum cultivars grafted on four rootstocks was studied over three years (2013-2015), while the total phenolic content and radical-scavenging activity in the skin and flesh were studied for only one year (2014).
The soluble solids were determined using a refractometer (Pocket PAL-1, Atago, Japan). Total sugar content was determined using Luff - Schoorl method. Total acids were determined by titration with NaOH and expressed as malic acid.
The extracts of plum skin and flesh were prepared according the previously described method (Pantelić et al., 2016). Frozen plums were used to separate skin from the flesh. The skin (approximately 3 g) and flesh (approximately 5 g) were extracted with 50 mL methanol containing 0.1 % HCl and stirred for 1 h on a magnetic agitator at room temperature. The extracts were left for 24 h in the dark at 4ºC, filtered, and the clear supernatants were

collected. The extraction step was repeated two more times and all supernatants were collected and evaporated to dryness under reduced pressure at 40ºC. The residue after evaporation was dissolved in a mixture of methanol/water (60:40, v/v) to 50 mL. All the extractions were performed in triplicate and the extracts were filtered through 0.45 μm membrane filters (Syringe Filter, PTFE, Supelco) before analysis.

The total phenolic content (TPC) in skin and flesh extracts was determined according the slightly modified method described by Singleton and Rossi (1965). Gallic acid was used as the standard in the concentration range of 20–100 mg L^{-1}. TPC values were expressed as milligrams of gallic acid equivalent (GAE) per gram of frozen weight (FW). $DPPH^{\cdot}$-scavenging activity of all extracts was assayed according to the method previously described by Pavlović et al. (2013). The Trolox calibration curve (100–600 μmol L^{-1}) was plotted as a function of the percentage inhibition of the $DPPH^{\cdot}$. The results are expressed as micromoles of Trolox equivalents per gram of sample (μmol TE g^{-1}).

Data of all measurements presented in the tables are the mean of three replicates ± standard deviation. Tukey's test was used to detect the significance of differences ($P \leq 0.05$) between mean values.

Results and discussion

According to many authors, the key parameters that determine the quality and the acceptance of the fruit by consumers are the content of soluble solids and total acids, as well as ration between them. (Nergiz and Yildiz, 1997; Crisosto et al., 2004). Soluble solids content of three plum cultivars grafted on four rootstocks ranged from 11.7 % to 14.2 % (Table 1). Highest values for soluble solids were found in 'Čačanska Najbolja', while the lowest values were found in 'Čačanska Rana' cultivar. However, differences among rootstocks were not significant. On the other hand, significant differences among cultivars were found. Also, significant differences for soluble solids were found between cultivar/rootstock combinations.

Table 1. Chemical composition of fruits of three plum cultivars grafted on four rootstocks (average 2013-2015).

Cultivar/rootstock combination	Soluble solids (%)	Total sugars (%)	Inverted sugars (%)	Sucrose (%)	Total acids (%)	Sugar/ acid ratio
Č. Lepotica/Fereley	13.0±0.3 de	10.6±0.6 c	7.6±0.6 a	2.9±0.4 bc	1.06±0.10 ab	10.0
Č. Lepotica/Myrobalan	13.8±0.6 abc	10.7±1.1 bc	7.4±1.0 ab	3.1±0.6 bc	0.98±0.03 bc	10.9
Č. Lepotica/Pixy	13.5±1.1 bcd	10.6±1.3 c	7.4±1.0 ab	3.0±0.6 bc	1.05±0.08 ab	10.1
Č. Lepotica/St. Julien A	13.3±1.1 cde	10.5±1.3 cd	7.5±1.2 ab	2.8±0.8 bc	1.06±0.14 ab	9.9
Č. Najbolja/Fereley	13.9±0.4 abc	11.5±0.6 a	7.4±0.9 ab	3.9±0.5 a	0.73±0.10c	15.6
Č. Najbolja/Myrobalan	14.2±0.6 a	11.4±1.1 a	7.6±1.0 a	3.6±0.4 a	0.75±0.06 c	15.2
Č. Najbolja/Pixy	14.1±0.7 ab	11.3±0.8 ab	7.5±0.9 ab	3.6±0.4 a	0.73±0.08 c	15.4
Č. Najbolja/St. Julien A	14.0±0.5 ab	11.3±0.7 ab	7.3±1.1 ab	3.8±0.4 a	0.71±0.09 c	15.9
Č. Rana/Fereley	11.7±1.2 f	9.5±1.2 e	6.6±1.2 c	2.7±0.3 c	1.06±0.07 ab	8.9
Č. Rana/Myrobalan	13.1±1.3 de	10.1±1.4 cde	7.1±1.6 abc	3.1±0.6 bc	1.11±0.07 a	9.1
Č. Rana/Pixy	12.8±1.2 de	10.4±0.9 cd	7.4±0.8 ab	2.8±0.3 bc	1.11±0.03 a	9.4
Č. Rana/St. Julien A	12.7±1.5 e	9.9±1.6 de	7.0±1.3 bc	2.7±0.6 c	1.06±0.05 ab	9.4
Rootstocks						
Fereley	12.9±0.3 b	10.5±0.7 a	7.2±0.9 a	3.2±0.4 a	0.95±0.08 a	11.0
Myrobalan	13.7±0.3 a	10.7±1.1 a	7.4±1.0 a	3.3±0.5 a	0.95±0.04 a	11.3
Pixy	13.5±0.7 a	10.8±1.0 a	7.4±0.9 a	3.1±0.4 a	0.96±0.05 a	11.2
St. Julien A	13.3±1.1 ab	10.6±1.3 a	7.3±1.2 a	3.1±0.5 a	0.94±0.08 a	11.2

Cultivars						
Čacanska Lepotica	13.4±0.7 b	10.6±1.3 b	7.5±1.0 a	3.0±0.7 b	1.04±0.09 a	10.2
Čacanska Najbolja	14.1±0.5 a	11.4±0.9 a	7.4±0.8 ab	3.7±0.4 b	0.73±0.08 b	15.5
Čacanska Rana	12.6±1.2 c	10.0±1.5 c	7.0±1.3 b	2.9±0.4 b	1.08±0.05 a	9.2

Means followed by the same letter in a column are not significantly different (Tukey's test, $p \leq 0.05$).

According to Milenković et al. (2006) early maturing plum cultivars developed at the Institute of Fruit Growing in Čačak, contain from 12.5 % to 14.8 % of soluble solids, while the medium and late maturing plum cultivars have from 16.8 % to 32.0 %. In the literature, the content of soluble solids in cultivars of European plum usually ranges from 16-18% (Miletić and Petrović, 1996; Oparnica and Jovanović, 2000; Nenadović-Mratinić et al., 2007). The content of soluble solids in a large number of cultivars in the conditions of the Belgrade region was in the interval from 12.3 % to 21.9 %, and these values were correlated with maturation time (Nenadović-Mratinić et al., 2007; Milatović et al., 2011, 2016, 2017, 2018).

One of the important parameters of quality of fruit is the content of total acids. The highest values of content of total acids were found in combinations 'Čačanska Rana'/Myrobalan and 'Čačanska Rana'/'St. Julien A' (1.11 %), while the lowest values were obtained in combination 'Čačanska Najbolja'/'St. Julien A' (0.71%) (Table 1). Differences in content of total acids among rootstocks were not significant, while the cultivar 'Čačanska Najbolja' had a statistically significantly lower value of content of total acids in relation to the other two cultivars. In the study of Dobričević et al. (2014), the content of total acids was lower, while the slightly higher value was observed by Miletić et al. (2005). The average content of total acids in the conditions of Belgrade for European plums was 0.9% (with variation from 0.5 to 1.9%) (Milatović, 2019). The acid content decreases rapidly after harvest, during storage of fruit (Milatović, 2019).

Total sugar content in plum fruits ranged between 9.5% ('Čačanska Rana'/'Fereley') to 11.5 % ('Čačanska Najbolja'/'Fereley') (Table 1). Analysis of data were not showed statistically significant differences in total sugar content among rootstocks. The highest average total sugar content was found in fruits from trees grafted on 'Pixy' rootstock, and the lowest were found in fruits from trees grafted on 'Fereley' rootstock. Cultivars manifested significant differences in total sugar content. The highest values of total sugar content (for all four rootstocks) were found in 'Čačanska Najbolja' cultivar, then in 'Čačanska Lepotica', while they were the lowest in 'Čačanska Rana' cultivar.

According Milenković et al. (2006) the total sugar content in the fruit of plum cultivars developed at the Institute of Fruit Growing in Čačak varied from 8.30% to 12.33%, while Milošević and Milošević (2012) established the interval of total sugar content 10.31-11.78 %. The results of our research of total sugar content were lower than that reported by Dugalić et al. (2014). The content of sugar in the fruit of plum depends of the cultivar (Nenadović-Mratinić et. al., 2007; Milošević and Milošević, 2011a; 2011b), temperature and the amount of rainfall in the period of maturation (Vangdal et al., 2007). In our study, total sugar content was the lowest in the earliest maturing cultivar, 'Čačanska Rana', and the highest in latest maturing cultivar 'Čačanska Najbolja'. All these findings confirmed the statements, that early maturing plum cultivars tend to have lower total sugar content in comparison with later maturing ones (Crisosto et al., 2007; Neumüller, 2010; Sahamishirazi et al., 2017).

The content of inverted sugars in the tested cultivars grafted on different rootstocks ranged between 6.6 to 7.6 % (Table 1). Significant differences of inverted sugars contents among cultivar/rootstock combinations were found. However, differences among rootstocks were not statistically significant. Cultivar 'Čačanska Lepotica' showed the highest amount of inverted sugars (7.5 %), followed by 'Čačanska Najbolja' (7.4 %) and 'Čačanska Rana' (7.0 %). According to Nergiz and Yildiz (1997) the content of inverted sugars varied between 37.6 % and 75.0 % in relation to the content of total sugars.

Sucrose content in plum fruits ranged between 2.7 % to 3.9 %. Differences among cultivars and rootstocks were not statistically significant. Among cultivars, the highest values of sucrose content were found in 'Čačanska Najbolja' (3.7 %), and the lowest values in 'Čačanska Rana' (2.9 %). Dugalić et al. (2014) reported that sucrose was the major sugar over the studied years in the tested cultivars, with a content of 4.52 %.

One of the good indicators of the overall quality of the fruit is the sugar/acid ratio, a common index for ripening and quality. The value of the sugar/acid ratio is higher in later maturing plum cultivars (Crisosto et al., 2004; Nenadović-Mratinić et al., 2007), but it also depends on the environmental conditions (Vitanova et al., 2007). The combination 'Čačanska Rana'/'Fereley' had the lowest value of sugar/acid ratio (8.9), while the combination 'Čačanska Najbolja'/'St. Julien A' had the highest value (15.9). Forni et al. (1992) reported sugar/acid ratio between 5.2 and 25.6.

Our study confirmed that soluble solids and total sugar contents are greatly affected by the cultivar. Namely, early maturing plum cultivars tend to have lower total sugar content in comparison with later maturing ones.

Total phenolic content (TPC) in the skin of plum fruits ranged from 4.44 to 15.93 mg gallic acid equivalent (GAE) g^{-1} FW, while in the flesh it varied between 0.38 and 1.52 mg GAE g^{-1} FW (Table 2). The highest average TPC values in the plum skin extracts were found in Myrobalan rootstock, and in the flesh in 'St. Julien A' rootstock. However, differences among rootstocks were not statistically significant. On the other hand, significant differences among cultivars were found.

Table 2. Total phenolic content (TPC, mg GAE g^{-1} FW), and radical-scavenging activity (RSA, μmol TE g^{-1} FW) in the skin and the flesh of three plum cultivars grafted on four rootstocks (2014).

Cultivar/rootstock combination	Skin		Flesh	
	TPC	RSA	TPC	RSA
Č. Lepotica/Fereley	4.44 ± 0.00 k	39.08 ± 0.79 i	0.72 ± 0.00 c	14.73 ± 0.03 c
Č. Lepotica/Myrobalan	4.89 ± 0.12 j	41.30 ± 0.29 h	0.57 ± 0.01 e	14.86 ± 0.02 c
Č. Lepotica/Pixy	6.30 ± 0.04 g	42.81 ± 2.67 gh	0.77 ± 0.02 b	14.73 ± 0.05 c
Č. Lepotica/St. Julien A	5.41 ± 0.05 i	43.89 ± 0.90 g	0.86 ± 0.01 a	16.97 ± 0.05 a
Č. Najbolja/Fereley	8.48 ± 0.03 e	57.60 ± 1.42 c	0.68 ± 0.01 d	14.89 ± 0.23 c
Č. Najbolja/Myrobalan	15.93 ± 0.11 a	78.49 ± 2.45 a	0.78 ± 0.01 b	13.49 ± 0.27 e
Č. Najbolja/Pixy	10.21 ± 0.10 c	52.54 ± 0.45 d	0.77 ± 0.03 b	15.32 ± 0.05 b
Č. Najbolja/St. Julien A	7.94 ± 0.05 f	50.77 ± 0.12 e	0.56 ± 0.01 e	13.41 ± 0.05 e
Č. Rana/Fereley	9.21 ± 0.10 d	47.70 ± 0.95 f	0.52 ± 0.03 f	10.40 ± 0.47 g
Č. Rana/Myrobalan	7.72 ± 0.09 f	57.39 ± 0.46 c	0.38 ± 0.02 h	11.00 ± 0.10 f
Č. Rana/Pixy	5.93 ± 0.09 h	44.38 ± 0.00 g	0.50 ± 0.01 g	13.13 ± 0.11 e
Č. Rana/St. Julien A	11.96 ± 0.29 b	62.70 ± 0.77 b	0.66 ± 0.00 f	14.26 ± 0.31 d
Rootstocks				
Fereley	7.38 ± 2.57 a	48.13 ± 9.26 a	0.64 ± 0.17 a	13.34 ± 2.55 a
Myrobalan	9.51 ± 5.73 a	59.06 ± 18.65 a	0.58 ± 0.21 a	13.12 ± 1.96 a
Pixy	7.48 ± 2.37 a	46.58 ± 5.22 a	0.68 ± 0.15 a	14.39 ± 1.13 a
St. Julien A	8.44 ± 3.30 a	52.43 ± 9.55 a	0.69 ± 0.15 a	14.88 ± 1.86 a
Cultivars				
Čacanska Lepotica	5.26 ± 0.80 b	41.76 ± 2.06 b	0.73 ±0.12 a	15.32±1.10 a
Čacanska Najbolja	10.64 ± 3.65 a	59.85 ± 12.76 a	0.70 ± 0.12 a	14.28 ±0.97 ab
Čacanska Rana	8.71 ± 2.55 ab	53.04 ± 8.48 a	0.52 ± 0.11 b	12.20±1.81 b

Means followed by the same letter in a column are not significantly different (Tukey's test, $p \leq 0.05$).

As for the plum skin extracts, highest values for TPC were found in 'Cacanska Najbolja', and the lowest values in 'Cacanska Lepotica'. In the plum flesh extracts, the highest values for TPC were found in 'Cacanska Lepotica', then in 'Cacanska Najbolja', while the lowest were obtained for 'Cacanska Rana' cultivar. Significant differences for TPC were also found among individual cultivar/rootstock combinations. High variability in the range of TPC was reported in previous studies in European plums (Chun et al., 2003; Kim et al., 2003; Rupasinghe et al., 2006; Rop et al., 2009). Sahamishirazi et al. (2017) found that TPC in 178 European plum cultivars ranged between 38.4 and 841.5 mg gallic acid equivalent (GAE) 100 g^{-1} of fresh weight. However, the results of TPC reported in the literature are obtained analyzing the whole fruit, precisely edible part of fruit consisting of flesh and skin. In our research, flesh and skin were analyzed separately. Because of that, our results are not fully comparable with others. Our results confirmed the findings of Chun et al. (2003) that 'Cacanska Najbolja' is among cultivars with the highest TPC. According to Stacewicz-Sapuntzakis et al. (2001) the skin contains about five times more phenolic substances than the flesh. In our study, TPC values determined in the fruit skin extracts were 6-20 times higher than in the flesh extracts of the same cultivar/rootstock combinations.

The total antioxidant capacity determined by scavenging DPPH assay in the plum skin varied between 39.08 and 78.49 μmol Trolox equivalents (TE) g^{-1} FW, and in the flesh varied from 10.40 to 16.97 μmol TE g-1 FW. Significant differences in radical-scavenging activity (RSA) were found among cultivars and cultivar/rootstock combinations, whereas differences between rootstocks were not significant. The highest values for RSA in the fruit flesh and skin were found in 'Cacanska Lepotica' and the lowest in 'Cacanska Rana' cultivar.

Our results for RSA values were higher than the results obtained by Voća et al. (2009) for three European plum cultivars and for 14 red flesh Japanese plum genotypes reported by Cevallos-Casals et al. (2006). Values of RSA in the skin of the plum fruit were 3 to 6 times higher than in the flesh of the same cultivar/rootstock combinations. The reasons for this are higher values for TPC, and absence of anthocyanins in the flesh. Significant correlations were found between TPC and RSA both in the skin (r = 0.93) and the flesh of plum fruit (r = 0.80). Significant correlation between TPC and antioxidant activity of the plum fruit obtained in our study are in accordance with previous findings (Kim et al., 2003; Rupasinghe et al., 2006; Rop et al., 2009; Mihalache Arion et al., 2014).

Conclusion

Our study confirmed that chemical composition of plum fruits was more cultivar- then rootstock-dependent. The earliest maturing cultivar, 'Cacanska Rana' had the lowest contents of soluble solids and total sugars, whilst the latest maturing cultivar, 'Cacanska Najbolja' had the highest contents of these ingredients. Total phenolic content in the fruit skin was 6-20 times higher than in the flesh of the same cultivar/rootstock combinations. Similarly, radical-scavenging activity in the skin was 3 to 6 times higher than in the flesh. Significant correlations were found between total phenolic content and radical-scavenging activity both in the skin (r = 0.93) and the flesh of plum fruit (r = 0.80). These results are of interest, as the phenolic content of fruits can be related to their antioxidant activity and their health-promoting properties.

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PRINCIPAL COMPONENT ANALYSIS IN EVALUATION OF EARLY MATURING SOYBEAN GENOTYPES

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Abstract

Principal Component Analysis (PCA) is frequently used in breeding work that includes multiple data analysis. This method provides a graphic insight into relationships between traits and evaluates genotypes according to multiple traits. Study encompassed 14 soybean genotypes belonging to maturity group 00 (very early varieties) and of different geographical origin, maintained in soybean collection of Maize Research Institute "Zemun Polje". Field trials were conducted during two years at two locations, according to a RCB design with three replications. Genotypes were evaluated for 8 major agronomic traits: seed yield, seed number per plant, 1000 seed weight, number of nodes and pods per plant, plant height and protein and oil content. PCA biplot explained a large portion of the variance of standardized data (85.8%). Genotypes were mostly distributed close to the biplot origin, as intermediate for most of the traits. Biplot revealed genotypes of potential importance for different breeding goals. Three varieties achieved the highest seed yield grain yield due to the high number of pods per plant and moderately high 1000 seed weight, which could be used in breeding for seed yield. Three genotypes of very high protein content were observed, potentially useful in breeding for improved seed quality. One genotype had the highest 1000 seed weight, the protein content above the group average while it was intermediate in seed yield. Correlations among traits determined by PC biplot were in accordance with Pearson's correlation coefficients.

Keywords: *Evaluation, Agronomic traits, Correlations, PCA*

Introduction

Total soybean harvested area in Serbia is 196472 ha, with average yield 3,286 t/ha (FAOSTAT, 2018). Although the seed yield is one of the most important breeding goals in soybean breeding programs, released varieties have to meet the requirements of processing industry regarding chemical composition and technological quality of grain (Peric *et al.*, 2018). With increase in the sample sizes of breeding materials and germplasm accessions used in crop improvement programs, methods to classify and order genetic variability are assuming considerable significance (Iqbal *et al.*, 2008).Among established multivariate analytical techniques for germplasmclassification, principal component analysis (PCA) is widely used for identification of genotypes superior for traits of interest (Mohammadi and Amri, 2011) and visualization of genetic correlations among traits (Yan and Rajcan, 2002).The special role of biplot lies in possibility for identification of genotypes that are simultaneously superior for a number of agronomically important traits, especially those that are in soybean negatively correlated such as protein and oil content or seed yield and protein content (Peric, 2015).

The aim of this study was to reveal superior soybean genotypes that could be used as parents in future breeding program, examine the correlations between agronomically important traits and establish important selection criteria by applying the method of PCA.

Material and methods

The plant material for this study comprised 14 soybean accessions of the maturity group 00 (very early maturing genotypes) from soybean collection of Maize Research Institute "Zemun Polje", originated from different parts of the world. The field trials were conducted during two agricultural seasons (2011 and 2012), at two locations in Serbia (Zemun Polje and Pancevo). Standard agricultural practice was applied. The soil type was slightly calcareous chernozem. During the both research years, particularly in 2012., severe drought occurred, with total rainfalls significantly lower and average temperatures much higher than multiyear average. Experiments were organized according to randomized block design with 3 replications and two rows per genotype. The experimental plot size was 5 m^2. The samples were collected at the R8 stage, by random selection of30 plants per genotype. A total of 8 agronomically important traits were analyzed: *PH* –plant height, *NN* – node number, *PN* – pod number, *SN* – seed number, *TSW* – 1000 seed weight, *SYP* – seed yield per plant, *PROT* – protein content, *OIL* – oil content. Analysis of seedprotein and oil content(expressed as a percentage on a dry matter basis) was performed using grain analyzer Infraneo, Chopin Technologies®. In order to determine correlations between agronomically important traits,evaluate genotypes on the basis of multiple traits and identify potential breeding sources among evaluated genotypes, Principal Component Analysis (PCA) was performed (Yan and Rajcan, 2002).Relationships of traits displayed by PCA were compared to Pearson's correlation coefficients (Hadživuković, 1991).

Results and discussion

PCA biplot of 14 soybean genotypes from very early maturity group explained 85.8% of the variance of standardized data (Figure 1). Distribution of genotypes along the first axis (PC1)was mainly on the basis of *PROT*, *TSW*, *SYP* and *OIL*, while second axis (PC2) separated genotypes by *PH*, *NN*, *PN* and *SN*.

A group of genotypes with the shortest genotypic vectors stands out on the biplot, concentrated around the coordinate origin (Olima, Evrika, Agassiz, Maple Arrow, Maple Presto, PI 180 507, GI 291/70-79). These genotypes were intermediate for majority of the observed traits and represent the so-called average genotypes within the group. Varieties Korana, Ljuso and Krajina had the highest *SYP* due to a significantly higher number of pods and grains than the group average, and a moderately high value of *TSW*. Cultivar Krajina was at the same time intermediate for protein content, so this genotype can be used for breeding for seed yield, while maintaining the protein content at a satisfactory level. Genotype Mini soja produced the largest number of pods and grains per plant, but due to the extremely low *TSW*, it ranked among the genotypes of the lowest yield.

The presence of three outliers can be noticed on biplot – genotypes Mini soja, Canatto and Progres stood out for their very high protein content, but due to the low *OIL* and low *SYP*, the breeding value of these genotypes is negligible. Variety Kabott achieved the highest *TSW*, protein content above the group average and was intermediate in grain yield. This variety represents a potential source of germplasm for breeding for seed size and increased protein content while maintaining yield within the average genotypes of very early vegetation.

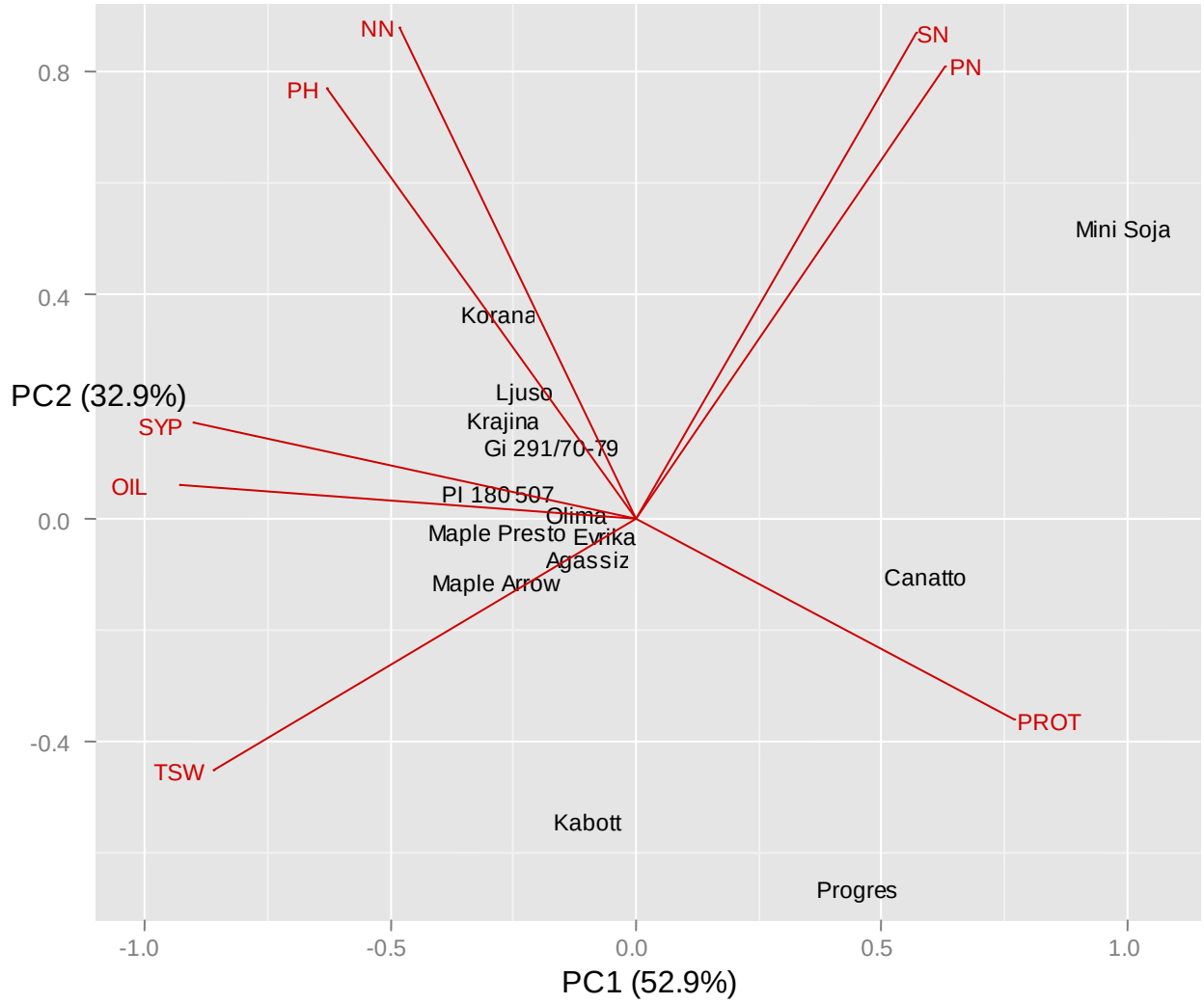


Figure 1PCA biplot of 14 soybean genotypes for 8 agronomic traits

PH – plant height, *NN* – node number *PN* – pod number, *SN* – seed number, *TSW*– 1000 seed weight, *SYP* – seed yield per plant, *PROT* – protein content, *OIL* – oil content

Correlations between traits displayed by trait vectors on biplot indicated that *SYP* had positive association with *TSW*, *NN*, *PH* and *OIL*, while it was negatively correlated with *PROT*, *SN* and *PN*. The correlations between the traits shown by the biplot were in accordance with Pearson's correlation coefficients (Table 1), both by vector directions (correlation sign) and by angles between vectors (correlation strength).

Table 1 Pearson's correlation coefficients between the traits

PH	0.87**	0.11	0.18	0.24	0.59*	-0.63*	0.57*
NN		0.25	0.32	0.082	0.50	-0.57*	0.44
PN			0.97**	-0.77**	-0.37	0.25	-0.52
SN				-0.78**	-0.30	0.18	- 0.45
TSW					0.79**	-0.41	0.66**
SYP						-0.64*	0.76**
PROT							-0.77**
	NN	PN	SN	TSW	SYP	PROT	OIL

PH –plant height, *NN* – node number *PN* – pod number, *SN* – seed number, *TSW* – 1000 seed weight, *SYP* – seed yield per plant, *PROT* – protein content, *OIL* – oil content

*P < 0.05; ** P <0.01

SYP was in a high positive and highly-significant correlation with *TSW* (0.79**), and consequently, a negative low non-significant correlation with *SN* (-0.30) and *PN* (-0.37). Furthermore, *TSW* was in negative and highly-significant correlation with *PN* (-0.77 **) and *SN* (-0.78 **). Similar correlations between seed yield and yield components are found by Pedersen and Lauer (2004), concluding that a high positive association of yield with seed size is accompanied by a negative association with the number of pods and seeds. Board *et al.* (1999) reported that *TSW* and *SN* were negatively correlated, and when influencing the yield, both components have the same indirect negative effect over each other, affecting the yield according to the principle of component compensation (Egli, 1994). A high and highly significant negative correlation was found between *PROT* and *OIL* (-0.77**), while *SYP* was negatively correlated with *PROT* (-0.64**) and positively with oil content (0.76**), confirming the results reported in numerous studies (Miladinovic *et al.*, 1996; Li and Burton, 2002; Taški-Ajduković *et al.*, 2010). *TSW* was in a positive moderately strong and highly significant correlation with the oil content (0.66 **) and a low negative non-significant correlation with the protein content (-0.41). Similar relationships between the three traits were found by Li and Burton (2002), suggesting that large seed selection could increase oil content without much reduction in protein content.

Conclusion

Biplot revealed genotypes of potential importance for different breeding goals. Three varieties achieved the highest grain yield due to the high number of pods per plant and moderately high 1000 seed weight, which could be used in breeding for seed yield. Three genotypes of very high protein content were observed, potentially useful in breeding for improved seed quality. One genotype had the highest 1000 seed weight, the protein content above the group average while it was intermediate in seed yield. Correlations among traits determined by PC biplot were in accordance with Pearson's correlation coefficients, indicating that 1000 seed weight could be important selection criterion in breeding for seed yield.

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VARIABILITY OF STEM HEIGHT IN WHEAT TRITICUM AESTIVUM L.

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Abstract

The stem height of wheat has impact on grain yield. Variability of height of wheat stem depends from environment and genetic structure of varieties. The aim of this study was estimation of impact of genetic components and environmental conditions on variability of stem height in 10 genetically divergent wheat varieties. The experiment was set up as a randomised block design in three replications. Obtained results indicated differences in average values of stem height among tested wheat varieties in each year of experiment. In the first year of study, the least stem height had Danica variety (44.87 cm) and the highest stem height had wheat variety Ljubičevka (64.73 cm. In the second year stem height varied between 64.37 cm in Danica variety and 87.97 cm in Šumadija variety. In average for all varieties the height of stem was higher in the second year than in the first year of experiment. Also, in average the value of height of stem was higher in second year than in the first year, in all analysed wheat varieties. The highest height of stem, expressed Šumadija variety in average (87.97 cm) in the second year. The least value of stem height (44.87 cm) in average had Danica variety in the first year of experiment. Based on the results significant differences between the wheat varieties according to stem height was established and impact of genetic factors prevailing compare to impact of environmental factors.

Keywords: *wheat, variability, height of stem, varieties*

Introduction

Stem height of wheat is component of plant architecture with connection to lodging of plant, variability of grain harvest index and grain yield. The resistance to plant lodging are determined by genetic factors and under influence of environmental factor (Zečević et al., 2005; Madić et al., 2016). In wheat breeding in period of "Green Revolution" breeders achieve introduce genes for reduction height of plant (dwarfing genes) which affected decreasing of stem height, increasing resistance to lodging of plant, increasing of grain yield and reduction of cost. The main role in stem reduction of wheat have *Rht-B1* and *Rht-D1* genes which response to gibberellin insensitivity and influence on number of grain and grain yield. The study of European recent winter wheat varieties showed that in the most the *Rht-D1* was significantly associated with plant height (Zanke et al., 2014; Würschum et al., 2015; Würschum et al., 2017). Also, other studies reported that dwarfing genes *Rht-B1b* and *Rht-D1b* are associated with Type I susceptibility to *Fusarium head blight* in wheat and low

anther extrusion (He et al., 2016). Numerous studies on *Rht* genes have concluded that *Rht8* gene reduces the plant height (Ellis et al., 2004; Rebetzke et al., 2011) but it has negligible effect on coleoptile length (Trethowan et al., 2001; Liatukas and Ruzgas, 2011; Grover et al., 2018). The wheat variety which carried *Rht-B1b* and *Rht-D1b* are not suitable for growing in area where heat and drought stress condition prevails during phase of grain filling (Ellis et al., 2005). However, wheat variety which carried combination gibberellic acid responsive *Rht8* gene and gibberellic acid insensitive (*Rht-B1b* and *Rht-D1b*) genes, are more adaptive on heat and drought conditions during phase grain filling and achieved higher grain yield (Rebetzke et al., 2011; Kaya et al., 2015). It mean than interaction of environmental factor and genotype affect expression of plant height in wheat varieties (Knežević et al., 1993). Also, technology measures of growing, as well application of different doses of fertilizer and plant growth regulators have influence on plant height (Spolidorio and Lollato, 2019). They reported that application of plant growth regulator (12% trinexapac-ethyl) during jointing phase, with standard dose of nitrogen fertilization, influence decreasing of wheat plant, in average 1,5 cm. The aim of this study was establish (i) variability of plant height in wheat varieties grown under different environmental condition of two years of experiment (ii) impact of genetic factor environmental factor and interaction of genotype/environment on plant height of wheat varieties.

Materials and Methods

This research encompasses 10 genetically divergent wheat varieties. Selected wheat genotypes were sown in field conditions in Kraljevo (city area in Serbia). Experiment was designed by randomized block system in three repetitions on plots of 1 m^2, in two growing seasons, (2015/16 and 2016/17). Sowing was done manually, by laying the seeds at a distance of 0.1 m in rows 1.0 m long, with a space of 0.20 m between rows. Sparse sowing was performed in order to enable the examined plants to fully manifest their traits. The plants were harvested for analysis in the phenology phase of full maturity. For all 10 wheat genotypes, 60 plants (20 plants per replicate) were used for stem height analysis. Mathematical-statistical analysis was performed, The analysis of variance (ANOVA) of monofactorial system examination by years was performed according by using the MSTAT C 5.0 version, The F-test was used to assess the significance of differences between average stem heights in the analyzed wheat genotypes. The LSD test was used for estimation significance of differences in values.

Weather conditions in the vegetation period

In the experimental year 2015/2016, the values of average monthly air temperatures and the total amount of precipitation per month differed in relation to the long-term period. In the first year of the experiment, the average temperature was 9.9 ° C and the total rainfall was 651.00 mm, which is significantly higher than in the second year in which the average temperature during the growing season was 8.7 ° C and the total rainfall 523.1 mm. In both years of the experiment with the varieties, the average temperature and the total amount of precipitation were higher in relation to the ten-year average (2000-2010) of the temperature values which were 8.5 ° C and 417.8 mm. For the development of plants, a more favorable temperature and precipitation regime was in the second year of cultivation. In the second year in the period October-November, the conditions for plant emergence were more favorable with the amount of precipitation of 161.7 mm, while in the first year in the same period there was a smaller amount of precipitation - 120.8 mm. In this period, the average temperature was higher by 0.8 °C in the first year, in which there was a smaller amount of precipitation, which could affect the slower emergence and development of plants in the crop. In the period February-March, in both experimental years, the average temperatures were similar but higher in

relation to the long term period average value, and there were also higher amounts of precipitation in relation to average of long term period. In the period February-April in the first year of the experiment, the amount of precipitation was 250.5 mm, which is higher than it was in the second year - 174.0 mm, but the distribution of precipitation was more favorable in the second year of the experiment. In the first year in April the average temperature was higher and the average amount of precipitation was lower than in the second and in relation to the multi-year period. In May, the temperatures were approximately the same in both years of the experiment and in the long-term period, while the amount of precipitation was significantly higher in the first year by 135.9 mm than in the second 100.0 mm and in both years significantly higher precipitation compared to the perennial period 52.6 mm.

Table 1. Average monthly temperatures and total monthly precipitation in Kraljevo
(*source: Republic Hydrometeorological service of Serbia)

Parameter	Period	Oct	Nov	Dec	Jan	Feb	March	April	May	June	Xm	Total
Temperature ^{0}C	2015/16	11,6	7,3	3,3	-0,1	8,8	7,8	14,1	15,5	21,3	**9,96**	89,64
Temperature ^{0}C	2016/17	10,6	6,8	0,0	-4,7	5,2	10,8	11,1	16,8	22,1	**8,74**	78,66
Temperature ^{0}C	2000-2010	11,8	6,4	1,7	-0,1	2,6	5,9	11,6	16,4	20,4	**8,5**	76,5
Precipitation (mm)	2015/16	56,8	64,0	9,0	86,2	52,7	157,9	39,9	135,9	48,6	72,3	**651,0**
Precipitation (mm)	2016/17	84,1	77,6	9,4	22,0	35,0	57,0	82,0	100,0	56,0	41,1	**523,1**
Precipitation (mm)	2000-2010	61,0	44,3	44,6	30,0	29,9	33,2	52,9	52,6	69,3	46,4	**417,8**

Results and Discussion

The height of the stem varied in the range from the lowest value - 44.87 cm in Danica, to the highest value - 64.73 cm in Ljubičevka in the first year of the experiment. The lowest stem height was 64.37 cm in Danica and the highest stem height was 84.33 cm in Ljubičevka in the second year of research. The average value of the height of a wheat stem in two years was the lowest 54.62 cm in Danica and the highest 74.53 cm in Ljubičevka. The average value of stem height for all wheat genotypes 53.4 cm in the first year was lower than in the second year 75.1 cm, while for all wheat varieties and in both years the height of the stem was 64.25 cm (Table 2).

Table 2. Variation of stem height (cm) in winter wheat varieties

Variety	Years		Average	Variety LSD		Year LSD		Variety x Year LSD	
	2015/16	2016/17		0.05	0.01	0.05	0.01	0.05	0.01
Fortuna	45.57lm	67.67gh	56.62FG	2.697	3.874	0.762	1.019	3.814	5.479
Sasanka	45.63lm	72.43e	59.03EF						
Danica	44.87m	64.37hi	54.62G						
Somborka	46.53lm	75.50de	61.02DE						
Kremna	49.07kl	68.33fg	58.70EF						
Kosmajka	52.50k	72.10ef	62.30D						
Šumadija	64.43hi	87.97a	76.20A						
Morava	59.00j	77.90cd	68.45C						
KG-56S	61.80ij	80.73bc	71.27B						
Ljubičevka	64.73ghi	84.33ab	74.53A						

Zečević et al. (2004) studied 50 wheat varieties from different selection centers in two years and found significant differences for plant height between years. In the first year plant height varied from 122,2 cm to 125,7 cm, while in second year plant height variate in ratio from 47,6 cm to 50.4 cm. The established that variability of plant height of wheat depends on

investigated variety and years and also, that significant differences between years according to values of plant height, indicated that two vegetation periods were ecologically different.
Analysis of variance showed that there were differences between wheat varieties for stem height, which were significant and highly significant. Differences between years for stem height in varieties indicate that there is an influence of environmental factors on the manifestation of plant stem height. In addition to this significance, the analysis of stem height variance for the total sample also revealed high significant differences for the values of the square mean for genotypes and for environments (weather conditions in the years).
The analysis of the components of variance for stem height confirmed that the environmental conditions in year had the greatest influence on the manifestation of stem height, 51.42%, while the share of genotype was 23.12% and the share of interaction was 4.24% (Table 3).

Table 3. Components of variance for stem height in wheat varieties

Sources of variation	DF	SS	MS	F-test	Probability	Components of variance	
						σ^2	%
Repetition	2	0.178	0.089	1.64322^{ns}	0.1755	-	-
Variety	9	3.352	0.372	7.4523**	0.0000	0,049	23,12
Year	1	3.023	3.023	64.2596**	0.0000	0,109	51,42
Variety x Year	9	0.588	0.065	1.6436^{ns}	0.1709	0,009	4,24
Error	38	1.746	0.045	-	-	0,045	21,22
Total	59	8.887	-	-	-	0.212	100,00

In similar studies conducted in a two-year experiment, was established the genetic factor had a share of 84.3%, environmental factors 7.5% and share of variety/year interaction was 6.7% in expression of plant height (Zečević et al., 2005). Similar studies conducted with bread wheat varieties showed a range of plant height variation between 66.5 cm and 112.2 cm and the average plant height for the examined varieties was 88.7 cm (Branković et al., 2015).

Conclusion

Based on the obtained results, the variation of stem height in the examined wheat varieties grown in two years, which differed according to weather conditions, was established. Varying the value of plant height in the same variety in two climatically different years shows the response of the genotype to the changed environmental conditions. The average value of the height of a wheat stem in two years was the lowest 54.62 cm in Danica and the highest 76.20 cm in Šumadija variety. The differences between genotypes were significantly high and highly significant for stem height. The expression of the studied trait was influenced by genetic factors, environmental factors and genotype/environment interaction. The largest share, 51.42%, on the height of the stem had environmental factors (years), and genotype.

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IMPACT OF ECOFRIENDLY RESISTANCE INDUCERS IN GREEN HOUSE AND FIELD TO CONTROLL BARLEY DISEASE

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Abstract

The antifungal activity of resistance inducers; Mono potassium phosphate (KH_2PO_4) ,chitosan clay Nano composite (ccnc), humic acid (HA) , Si was tested in controlling *Pyrenophoratere* Ana morph : (*Drechslera teres*) the causal organism of net blotch of barley .And leaf rust (*Puccinia hordei*) The evaluation was carried out at barley adult plant stages using two rowed hulled Giza 128and six rowed hulled Giza ,130 under green house. All concentrations of the four tested inducers were significantly and negatively correlated with each of the biochemical traits . The highest correlation was observed between DS and 1,3- glucanase after 15 days . and under open field conditions at two locations ; Giza and Sakha stations Giza and Kafr el Sheik Governorates , Egypt in 20 20 growing season .All concentrations of the four tested inducers were significantly and negatively correlated with each of tested inducers showed significantly correlation between disease severity and thousand kernel weight (TKW) , plot weight , total protein , total lipids , total fiber , crude ash and total carbohydrates . It is worth mentioning that decreased disease severity resulted significant increase of 1000 kernel weight (g) compared to the control. Stubble-borne diseases, spot form of net blotch (SFNB), net from of net blotch (NFNB) will be present in many paddocks due to carry-over from the last two seasons. Soil-borne diseases are a risk to barley .On other hand using four inducers decreased disease severity and safety they have no toxic residues and environmental friendly and coast effective compared with the ordinary fungicides. An explanation of the resistance ratings used in this guide for foliar diseases , and how they should be interpreted (R) Resistant , (MR) Moderately Resistant , (S) Susceptible , (VS) Very Susceptible .

Keywords: *Barley, Antifungal activity, Biological control, Inducers, Egypt*.

Introduction

Barley (*Hordeum vulgare* L.) is an important cereal grain crop, that is grown all over the world . Which ranks fifth globally among all crops in dry matter production (Baik and Ullrich, 2008). It is mainly used as human food and in malt industries, but there is a growing interest in it for animal feed (Bergh *et al.*, 1999).

In Egypt barley is the main crop grown in a large scale in the North Costal Region and also in the newly reclaimed lands with saline soils and shortage of fresh water. The total harvested area was 57.000 ha in the eighties, while it increased to 135.000 ha in 2008/2009. Barley yields have also increased gradually over the past three decades from 2.92 $t.h^{-1}$ in the eighties to 3.63 $t.h^{-1}$ in 2008/2009 (El-Banna *et al.*, 2011).The disease is associated with high loses (70-80%) in humid regions (Cherif *et al*, 1994). Net blotch is a seed-borne and a foliar disease of barley (Douglas and Gordon, 1985) caused by the fungus *Drechslera teres* (Sacc.) Shoem. .The disease occurs in two forms; net form of net blotch (NFNB, *D. teres* f. *teres*) and spot form of net blotch (SFNB, *D. teres* f. *maculata*) (Hollaway and Mclean, 2008).The

optimum conditions for infection are from 10 to 30 hours of high humidity and a temperature from 15-25°C (Sato and Takeda, 1990).,

Barley leaf rust (caused by *Puccinia hordei*)(LR) is one of the most important barley disease worldwide . in experimental conditions ,yield losses as high as 60% can happen in highly susceptible barly cultivars, but losses of about half that level are common in practice (Das *et. al.* 2007) . Resistance breeding can be the economically and environmentally effective strategy to reduce the yield losses caused by rust.

The objective of the present was to investigate the effect of some safe resistance inducers for controlling the Barley Net blotch disease and leaf rust disease under greenhouse and field conditions

Materials and Methods

Fungal pathogen: Pathogenic isolates of *Drechslerateres* isolated early from infected barley leaves typical net like lesions and used for all experiments in this study . Barley plants were regularly inoculated with a mixture of spore and mycelium fragments prepared from slant culture

Source of seed and plant growth : (Giza 128) as two rowed hulled and (Giza 130) as six rowed hulls from Barley Research Section field crop Institute, Agriculture Research Center (ARC) Giza , Egypt .The following experiments were conducted in the greenhouse , laboratories and field

Greenhouse experiments:

Effect of four compounds as resistance inducers .Four compounds ; Mono potassium phosphate (KH_2PO_4) (50 &100µg/ml) , chitosan clay Nano composite (ccnc)(15 &30 mg/ml) , Humic acid(1.5 & 2.5 m/l) ,Sodium meta silicate(Si) (1& 1.5 g/l) , were tested to evaluate their capabilities to induce resistance against net blotch (BNB) caused by *pyrenphorateres*Ana morph : *Drechslerateres.* And leaf rust caused by *(puccinia hordei),*The experiments were carried out on seedling and adult plant stages using (Giza 128) as two rowed hulled and (Giza 130) as six rowed hulls . Mono potassium phosphate (KH_2PO_4), chitosan clay Nano composite and Humic acid,were obtained from central Laboratory of Organic Agriculture , Agriculture Res. Center ,Giza , Egypt .Sodium meta silicate (Si) was obtained from Sigma Aldrich Chemical Co. (St. Louis, MO, USA) . Barley grains were obtained from the Barley Research Section , Crops Research Institute , Agriculture Research Centre ,Giza , Egypt .Barley plants were grown in the clay pots (15 cm diameter) in a greenhouse at 20±2ºC for 2 weeks for the development of net blotch , plant seedlings at the 3rd leaf stage were inoculated with *Drechslera teres.*by spring a spore suspension of the fungus adjusted to a concentration of 2×10^4 conidia / ml

(S Gupta et al 2003) (M Jalli et al 2011).The inoculated plants were incubated in the dark for 48 h at 20±2ºC and 100 % relative humidity . they were then transferred to a growth chamber maintained at 20±2ºC and 70-80 % RH with 12 hr daylight per day . Disease severity was determined as the percentage of infected leaf area 9 days after inoculation.On the other hand seedling of (Giza 128) as two rowed hulled and (Giza 130) as six rowed were inoculated with leaf rust isolate Fresh urediospores were diluted ten times with lycopodium spores and dusted over the adaxial sides of seedling leaves fixed in a horizontal position . After incubation at a relative humidity of 100% over – night, the seedling were transferred to a greenhouse where the temperature was set at about 18 ºC . The latent period (LP) of each plant was evaluated by estimating the period at which 50% of the ultimate number of pustules became visible.

Adult plants in the greenhouse:
The rust isolate was also used for evaluation of the RILs in the adult plant stage in the greenhouse. One experiment was carried out with 5 plants per line.
Disease assessments at seedling stage: Disease assessments of BNB were carried out 10 d after inoculation (DAI). The net-like necrosis was assessed visually using the rating scale of Tekauz (1985).They should be interpreted (R) Resistant, (MR) Moderately Resistant , (S) Susceptible , (VS) Very Susceptible
Statistical analysis: Data were analysed with the statistical analysis system SAS. All multiple comparisons were first subjected to analysis of variance (ANOVA) comparisons among means the least significant differences (LSD) AT P =0.05 and Duncan's multiple rang test (Duncan, 1995) was obtained .

Result and Discussion

Table (1) Analysis of variance of disease severity (DS) when Mono Potassium Phosphate(KH_2PO_4) ,Chitosan nanoparticles , Humic acid and Silicon applied at three concentration on barley cultivars

Source of variation	D. F.	M.S.	F.VALU	P > F
Concentration (c)	2	4.709	20.0345	0.0000
Inducer (I)	3	1.480	6.2943	0.0006
C X I	6	0.488	2.0745	
Variety (v)	3	1.335	5.6784	0.0013
C X V	6	0.210	0.8933	
I X V	9	0.147	0.7408	
C X V X I	18	0.069	0.2919	
Error	96	0.235		

Table (2) correlation between disease severity (DS) and biochemical traits under green hose condition

	Variable									
Variable.	1	2	3	4	5	6	7	8	9	10
Free ph3day										
Total ph 3 day	0.653**									
Free ph.15day	0.797**	0.603**								
Totalph.15day	0.748**	0.706**	0.824**							
Ppo 3 day	0.613**	0.491**	0.519**	0.429**						
Ppo 15 day	0.598**	0.460**	0.513**	0.465**	0.878**					
Peroxidase 3	0.634**	0.432**	0.561**	0.556**	0.693**	0.694**				
Peroxidase 15	0.622**	0.483**	0.570**	0.577**	0.704**	0.793**	0.827**			
β1,3-glucanas3d	0.458**	0.349**	0.433**	0.386**	0.601**	0.642**	0.509**	0.595**		
β1,3-glucanas15	0.485**	0.358**	0.474**	0.450**	0.634**	0.711**	0.509**	0.628**	0.841**	
D.S	0.398**	0.407**	0.361**	0.466**	0.350**	0.473**	0.321**	0.351**	0.343**	0.531**

Field experiments **:**

Table (3) correlation between disease severity , agronomic traits , technological traits under field condition in Giza

No.		Variable						
	variable	1	2	3	4	5	6	7
1	1000 kernel weight							
2	plot weight	0.539**[a]						
3	total protein	- 0.067	-0.435**					
4	total lipids	0.058	-0.011	0.558**				
5	total fiber	0.256**	-0.039	0.797**	0.836**			
6	crude ash	- 0.012	-0.253**	0.790**	0.721**	0.844**		
7	total carbohydrates	-0.138	-0.093	0.221**	0.819**	0.475**	0.498**	
8	disease severity	-0.648**	-0.493**	-0.061	-0.127	-0.362**	-0.035	0.112

(r) is significant at $p \leq 0.01$ (* *) and n =48

Table showed highly significant correlation between disease severity and each of 1000 kernel weight , plot weight , total protein , total lipids , total fiber , crude ash, total carbohydrates . The negative correlation between disease severity and 1000 kernel weight implies that the substance , which significantly decreased disease severity will induce significant increase in 1000 kernel weight

Table (4) correlation between disease severity , agronomic traits , technological traits under field condition in Sakha

No.		Variable						
	variable	1	2	3	4	5	6	7
1	1000 kernel weight							
2	plot weight	0.739**[a]						
3	total protein	0.089	-0.005					
4	total lipids	0.183*	0.144	0.580**				
5	total fiber	0.355**	0.282**	0.789**	0.862**			
6	crude ash	0.053	0.004	0.784**	0.724**	0.835**		
7	total carbohydrates	-0.106	-0.171*	0.224**	0.807**	0.508**	0.502**	
8	disease severity	-0.716**	-0.688**	-0.400**	-0.263**	-0.501**	-0.243**	0.124

A linear correlation coefficient (r) is significant at $p \leq 0.01$ (* *) and n =4

disease severity (DS) was significantly and negatively correlated with each of the biochemical traits . the highest correlation was observed between DS and 1,3- glucanase after 15 day.

Mono potassium phosphate (KH_2PO_4) (50 &100µg/ml) , chitosan clay Nano composite (ccnc)(15 &30 mg/ml) , Humic acid(1.5 & 2.5 m/l) ,Sodium meta silicate(Si) (1& 1.5 g/l) reduced the injurious effect of net blotch (BNB)and leaf rust(LR) on barley Mono potassium phosphate followed by chitosan clay Nano composite the most effective in reducing barley infection by net blotch . in addition , all of treatments used enhanced plant growth productivity.In the present study , in greenhouse condition all the treatments significantly reduced disease severity compared to control (untreated). Induced resistance against net blotch disease and leaf rust(LR) infection on barley by using the Mono potassium phosphate, chitosan clay Nano composite, Humic acid and Sodium meta silicate due to the increasing the activities of β – 1,3gluconase(Schneider and ultrich ,1994), peroxidase and Poly phenol oxidase (Hiraga et al.,2001).Efficacy of KH_2PO_4 against plant infection to trigger plant defense through a process involving the consequent of calcium from host issue , elicits the

release of signal triggers of plant response and makes the plant more resistance and makes the plant more responsive after subsequent infection .(Gottstein and Kuc, 1989 and Mucharromah and Kuc, 1991) . Moreover KH_2PO_4Led to increase synthesis of host metabolites such as phytoalexins and their production could be induced by many chemicals and increase of enzymes activities and total phenols (Nighat et al., 2011) .

Conclusions

Finally , obtained results and discussion concluded that the application of chemical inducers could be used as a safe and cost effective method for controlling net blotch and leaf rust (LR) disease as well a suitable for integration in disease control system as they have no toxic residues and environmental friendly and coast effective compared with the ordinary fungicides.

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COMPARATIVE EXPERIMENT OF SEVERAL EARLY SWEET CORN VARIETIES

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Abstract

According to data, sweet corn is the vegetable which is grown on the greatest area in Hungary. The aim of paper was to evaluate some important morphological properties of several early sweet corn varieties for fresh consumption market, in low cost technology for small producers. The experiment was set up in 2016 in Tg-Mures, Mures County, situated in Central part of Romania. The choosen varieties: a conventional, very early ripening, normal sweet hybrid, „Spirit" which is used as a reference variety in the variety comparison trials conducted by Central Agricultural Office, then early ripening, normal sweet hybrid, „Mv. Július" and early ripening, super sweet hybrid „Sweet Star". The compared varieties could have been recommended as adaptable for meteorological and pedological conditions of Central part of Romania. No growing period shortening technologies were applied but direct sowing of plants without row cover, normal period (third decade of April). Results showed the influence of variety on some important morphological properties of sweet corn ears: weight of husked and unhusked ears, ear length, ear diameter, length of kernel.

Key words: *earliness, sweet corn, normal sweet, super sweet*.

Introduction

Based on its present growing area, the sweet corn is the vegetable which is grown on the greatest area in Hungary. According to data of Hungarian Fruit & Vegetable Interprofessional Organization since 2005 the growing area was about 30,000 hectares. Sweet corn (Zea mays L. convar. saccharata), considered a vegetable, because of is destined exclusively for human consumption, less (2-3%) in fresh form or more (97-98%) in processed (canned or frozen) foods (Riverti *et al.*, 2018). Near 50% of world sweet corn production came from US (Slezák *et al.*, 2012).

In order to promote fresh consumption, as well as to maintain and increase the sweet corn exports, it is necessary to promote investigations so as to be able to ensure a further increase in the growing area and yields of sweet corn with the help of new experiences. The references mainly are concerned with maize growing technology, but a lot of dates could be used by sweet corn growers. Among production technology elements, a number of researchers studied or are currently studying the sowing time of sweet corn, one of important factors which influence both the ear yield and ear quality (Rosa, 2014).

As early as at the ending of 19th- and the beginning of the 20th century some researchers (Cserháti, 1901) highlighted the importance of the sowing date. At second half of 20th century more other researchers mentioned the importance of the sowing date. Ripening can occur earlier when sowing earlier and using high quality seeds as compared to normal or late sowing (Elshatoury and El-Sayed, 2014). I'só (1969) and Pásztor (1966), after their multi-year sowing date trial, concluded the following: in the case of an earlier sowing seed germination will be more protracted, but silking and harvesting occur sooner than by lately sowing time. After multi-year trial Berzsenyi et al (1998) had studied the effect of different sowing times on maize development.

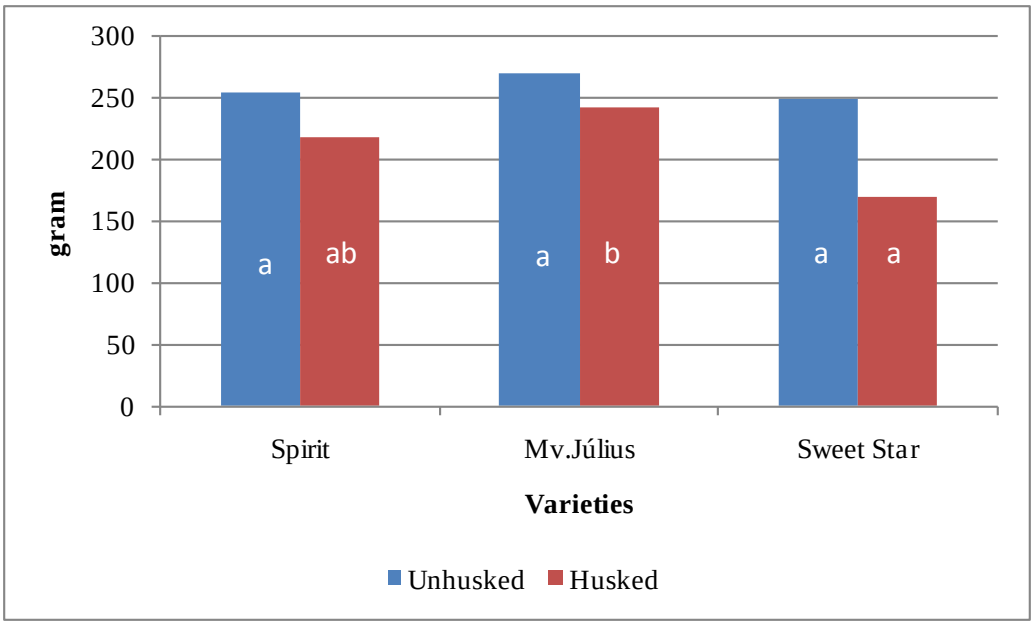
About the covered early sowing as a technological variation Aylswirth (1986) mentioned, that from an early sowed crop, made in first week of April, arranged in twin rows (42 cm) and covered by plastic, we could harvested marketable cobs by the fourth of July.
Objective of this trial was to determine more early ripening sweet corn hybrids for Central Transylvanian conditions.

Materials and methods

The experiment was set up in 2016 on an area eqiupped for irrigation at Tg-Mures, Mures County, situated in Central part of Romania. Conventional, reliable and sufficiently known among growers normal sweet corn variety, Spirit, was used as a reference variety in the variety comparison trials of the Central Agricultural Office. Hybrid has very short growing period of 85 days and yellow kernels. Average height of plants 159 cm, average ear height is 37 cm, ear length 19.6 cm and average ear weight is 245 g. The variety was granted official recognitions in 1988 and has been the dominant variety of the early ripening category till now.
Mv.Júilus (normal sweet) also very short growing period, 85 days same period compared o Spirit and yellow kernels. Average height of plants 160 cm, average ear height is 35 cm, ear length 20.8 cm and average ear weight is 240 g.
Sweet Star (Syngenta), super sweet corn variety, very short growing period 87-88 days and yellow kernels. Average height of plants 210 cm, average ear height is 42 cm, ear length 19 cm.
In the year prior to the experiment the area was under wheat.
Crop density 62,111 plants per hectare, according to the recommendations of the owner of the variety, at a spacing of 70x23 cm in single rows. Each plot had an area of 6x3.5m (8 parallel rows and 16 seeds sown in each row). Sowing date in same time for all varieties 24th of April, sowing depth was 3 cm. Harvesting time 17th of July.
In October 2015, 35 t/ha of farmyard manure was applied into the soil with ploughing on area. Nitrogen fertilizer (120 kg/ha) was applied at the 6-7 leaf stage, the form of top dressing. Fertilizer application was worked into the soil with a rotary hoe.
For measurements, were selected 10 plants/treatment/replication, from the four central rows.
Ears were harvested, from selected plants, together with husks, from the two central twin rows and the following measurements were carried out:
weight of unhusked and husked ears (g),
total ear length (cm),
length of kernels (mm),
ear diameter (mm).
The statistical analysis of the results was carried out by using the programme *RopStat 1.1*. When the standard deviations were identical the mean values were compared by pairs using the *Tukey-Kramer* test, while in the case of the non identical standard deviations the means were compared using the *Games-Howell* test (Vargha, 2007).

Results and discussion

Results of the one of the major characteristics in connection with yield rating, unhusked and husked ear weight, are summarised in Figure 1.



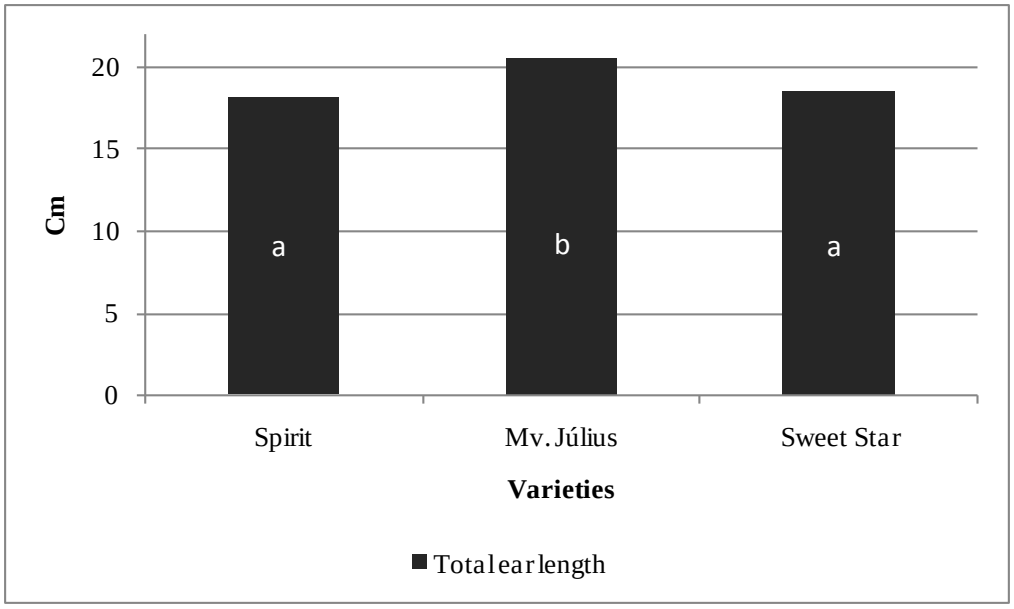
F(3;163)=23,155; F(3;163)=13,772;Sd=99%

Fig. 1. Unhusked and husked ear weight (g).

Comparing measured results for unhusked ear yield, it is observable that the average weight of the ears of studied varieties, presented some differences, the highest value presented Mv. Július. Though there was some difference between the unhusked ear weight compared to Spirit and Sweet Star varieties, the fact statistically was not significant.

In case of husked ear weight the same trend could be notified, Mv. Július produced the highest value among the varieties. Husked ear weight result higher compared to Spirit, but not significantly, and was significantly (at $p<0.01$ level) higher compared to Sweet Star variety.

The data concerning, an important characteristics for market appeal (total ear length) are contained in Figure 2.



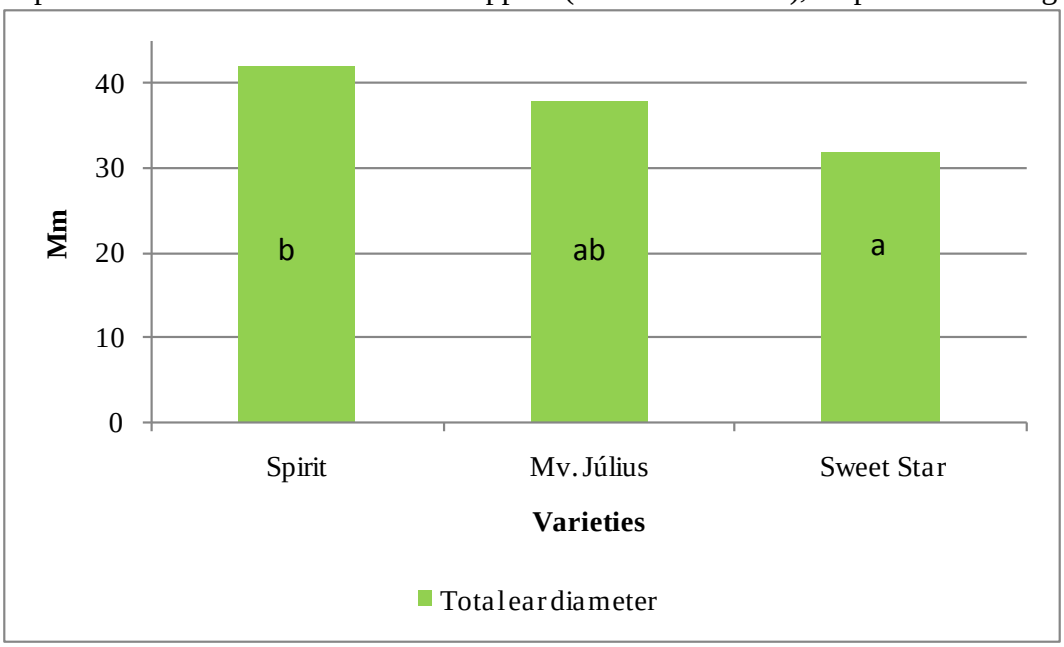
F(3;163)=10,000, Sd=99%

Fig. 2. Total ear length (cm).

The length of Mv.Július variety greater compared to ear length of Spirit and Sweet Star varieties. The measured values were significantly (at $p<0.01$ level) higher.

Total ear length, average ear length as measured in the variety comparison trials, had been not achieved by the above mentioned varieties, compared to official description, in year of this trial.

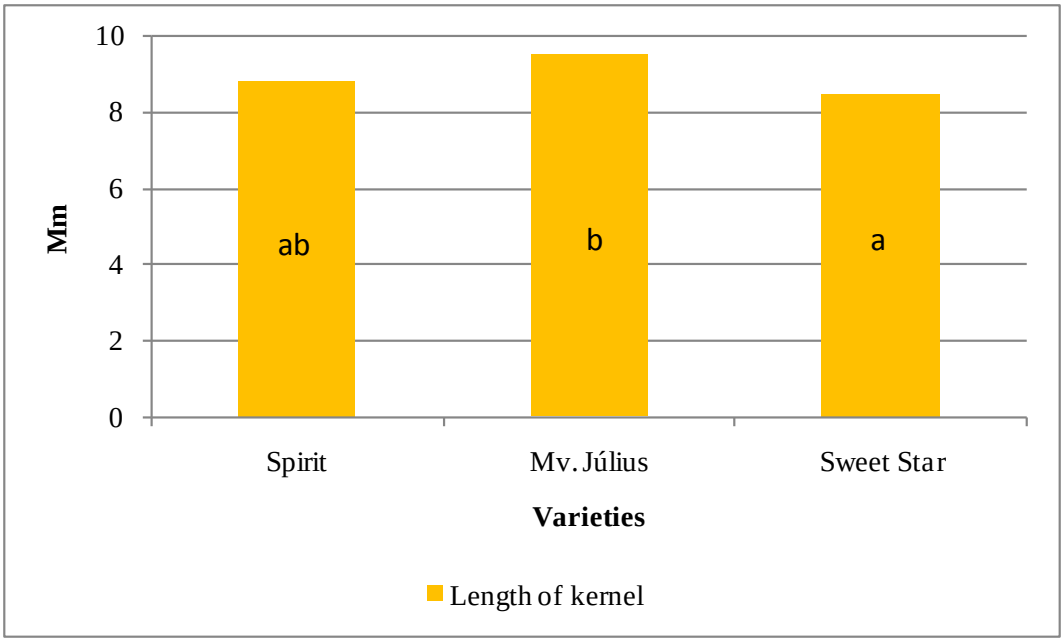
Other important characteristics for market appeal (total ear diameter), is presented in Figure 3.



F(3;163)=3,420, Sd=99%

Fig. 3. Total ear diameter (mm).

The measured values were significantly (at $p<0.01$ level) higher in case of Spirit variety compared to Sweet Star and higher, without statistical demonstrable difference compared to Mv. Július. Total ear diameter of Sweet star and Mv. Július presented no significant difference. Length of kernel, an important characteristic for yield quantity, is presented on Figure 4.



F35;163)=1,146, Sd=99%

Fig. 4. Length of kernel (mm).

The measured length of kernels values were significantly (at $p<0.01$ level) higher in case of Mv. Július compared to Sweet Star and higher, without significancy, compared to Spirit. Length of kernels of Spirit variety higher than Sweet Star, no significantly difference was found.

Conclusions

Based on the results of the 2016 year experiment, the following conclusions can be made:
Growing period was shorter as variety owners description, 1 day in case of Spirit and Mv. Július varieties, and 3 days by Sweet Star variety.
The mentioned varieties presented interest among growers in consideration of earliness, and are able for further experiments.
From point of view, of important morphological properties for example unhusked and husked ear weight, Spirit and Mv. Július, evaluated varieties, presented same results as in oficial description.
Mv. Július presented, significantly (at $p<0.01$ level), higher result compared to Spirit and Sweet Star varieties, in csae of total ear length and length of kernels.
The measured total ear diameter values were higher in case of Spirit, compared to Mv. Július and Sweet Star varieties.
Obtained results represents effect of 2016 experimental year, experiment should continue for further results.

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DETERMINATION OF PHENOLOGICAL AND GENETIC VARIATIONS IN WALNUT SEEDLING GENOTYPES

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Abstract

This research was carried out to determine phenological and genetic differences of the genotypes obtained from the free dusting in the Maras 18 walnut cultivar and the main cultivar (Maras 18). In order to determine the phenological differences between the main plant and genotypes, traits such as budburst, first foliation, leaf yellowing and defoliation dates periods were investigated. 12 ISSR primers were used to determine genetic variations. Among the phenological characteristics examined in the study, the budburst, first leafing, leaf yellowing and defoliation dates periods were found 45 days, 45 days, 31 days and 43 days, respectively. The variation rate between the main cultivar and genotypes was 96.25% for bud burst, 93.75% for the leafing, 100% for leaf yellowing and 97.5% for defoliation, while the average phenological variation was 96.87%. From the genetic analysis, 29 bands were obtained in total 26 of these bands (89.6%) were polymorphic with the average number of alleles of 4.33. Most bands obtained from ISSR 5 and ISSR 12 were primer. According to genotypes, Polymorphism Information Content (PIC) value ranged from 0.21 to 0.98, and the mean PIC value was calculated as 0.63. From clustering analysis, the genotypes were clearly divided into 2 main clusters. This present research indicated that the genotypes had phenologically and genetically important variations when compared with the main cultivar. The results also showed that the use of phenological and molecular data together in the management of gene resources were important. In addition, ISSR marker techniques were confirmed as an effective method to determine genetic differences in walnut genotypes.

Key words: *Walnut, Juglans regia L., phenological, molecular, ISSR.*

Introduction

Walnut is a type of plant that can grow up to 25-30 meters and generally has a broad crown trees structure. Branching usually starts at a height of 1-2 meters above the soil level. Shoots are glabrous and light gray, dark gray, or black in colour. On these shoots, leaves and fruits are in order, arranged mutually. Male flowers occur on annual shoots, while female flowers are formed on the shoots of the same year. Walnut has a monoic flower structure. Pollination occurs via the wind. Walnut has a large number of chromosomes (2n=32) (Şen, 2011; Özcan and Sütyemez, 2017). According to the statistics from 2018, worldwide walnut production was 5,248,874 t. Among the most important walnut producing countries in the world, shelled walnut production is 1,586,367 t in China, 613,260 t in the USA, 409,562 t in Iran, and 215,000 t in Turkey (FAO, 2020). The heterozygous structure of fruit species such as walnuts restricts the reproduction of these fruit species with seed. Although this situation creates a negative effect in terms of production, it provides an important genetic variation for plant breeders. However, the long period of youth infertility in walnuts is another important problem that restricts breeding activities. The rapid progress in biotechnological methods in recent years has made significant contributions to shortening this period. With the help of

molecular markers, DNA part of related to any gene region are replicated in the genome. These marker technologies are used effectively in many areas such as physical mapping, gene discovery, and labeling, phylogenetic studies, genetic diversity studies (Filiz and Koç, 2011; Özcan et all. 2020). In this study, it was aimed to determine the similarity within offspring genotypes and with the main plant in terms of some phenological properties and describing and categorizing genetic relations between them using the ISSR marker technique.

Material and Methods

In this study, the main (parent) variety Maraş 18 and 80 genotypes that were obtained from its free-pollinated seeds were used as the material. In the study, the characteristics like leaf out and defoliation onset were determined according to the parameters given in IPGRI to identify the phenological differences of the walnut varieties (1994) (Table 1).

Table 1. Definitions used in determining the phenological characteristics

Characteristics	Definitions
Bud break date	Full visualization of the green areas from below the bud flakes
Leafing date	Date of breaking 50% of the top buds and visualization of green leaves
Date of yellowing in the leaves	Date on which the green color turned into yellow completely over time
Date of defoliation	Date on which all of the leaves on the plant were lost

Source: IPGRI, 1994

DNA isolation and Extraction; Walnut DNA was isolated using the CTAB protocol (Doyle and Doyle, 1987), modified by Bardak (2012). Young and healthy leaves were used for DNA extraction. The collected leaves were kept in dry ice, transferred to the laboratory and then stored in at -80□ for DNA isolation. Genomic DNA was extracted from approximately 0.5 g of young, fresh walnut leaves by using the optimized Cetyl Trimethyl Ammonium Bromide (CTAB) method as described by Doyle. DNA concentration by spectrophotometry (SPECTROstar Omega) were standardized to 2.5 ng/µL and visualized on 1% agarose gels stained with ethidium bromide. ISSR-PCR Amplification; For the purpose of determine the genetic differences between walnut genotypes, ISSR primers used by Bardak ve Bölek (2012) were employed. PCR reactions were performed in a total 20 µl volume. The reaction mixture contained 2 µl 10X PCR buffer, 1 µl dNTP (25 mM), 1.5 µl MgCl2, 1 µl ISSR primer, 2 µl genomic DNA (25 ng), 0.5 µl DNA Taq polimeraz (vivantis), 12 µl dH_2O. The PCR temperature reaction was carried out as follows: initial denaturation for 5 min at 94□, followed by 35 cycles of denaturation of 1min at 94□, annealing for 1min at 55□ (based on the used primer) and extension for 1 min at 72□, followed by a final extension of 10 min at 72□. The gel images were then visualized by UV light and recorded. Data Analysis; The ISSR PCR product was run in an electrophoresis gel. The gel images were analyzed and data were scored as 1 for the presence and 0 for the absence of the products (Laborda et al., 2005). Polymorphism information contents of the primers were used in this study and calculated by the Excel software. Total polymorphic bands were calculated and allele frequencies of each band were calculated according to the following formula. Here, *f* i denotes the frequency of the 'i' band. **PIC=1-∑ $(f_i)2$** The coefficient of similarity between walnut genotypes was calculated in the POPGENE 3.2 version 1.2 package program (Nei, 1972). The unweighted pair group with arithmetic means (UPGMA) entered the clustering method using NTSYSpc software (Ver. 2.02), (Rohlf, 1998). Cluster analysis of walnut genotypes was done by the Structure 2.3.4 package program (Pritchard et al., 2000). To determine the ideal number of groups, each K value was run from 1 to 10 with 10 independent simulations. The permutation module was chosen between 10,000 and 100,000. Delta K value, which determines the

number of groups, was calculated by making 5 repetitions for each K value (Evanno et al., 2005). The results of the analysis were uploaded to the 'structure harvester' (Earl and VonHoldt, 2012) web page and the ideal ΔK value was found (Anonymous, 2020).

Results and Discussion

In the research, phenological observations and molecular analysis were carried out on genotypes in accordance with the method. The findings obtained as a result of the study are presented below. It was determined that the bud burst in the main variety took place on March 16. It was determined that the bud burst date of all genotypes were spread over 45 days. In this aspect, it has been determined that 3 genotypes out of 80 genotypes studied are the same as the main variety, 1 genotype is before the main variety, and the remaining 76 genotypes begin to bud burst later than the main variety. It has been determined that walnut plants in the population of this study have high degrees of variation in terms of the bud burst (Table 2). In the 80 genotypes, leafing spanned through 45 days, while leafing occurred 26 March in the main variety. In this aspect, it has been determined that 5 genotypes out of 80 genotypes studied are the same as the main variety, 1 genotype is before the main variety, and the remaining 74 genotypes begin to leafing later than the main variety. According to results, it determined that the phenological variation between the main variety and genotypes in terms of this property (Table 2). It is given in the study results of different researchers that the leafing periods of the Maraş 18 walnut varieties are between March 4 and March 22 (Sütyemez and Kaşka, 2002). In this study, it is seen that the bud burst of the Maraş 18 walnut variety started on March 16. Accordingly, it appears that there is a similarity between our research and other studies. It was observed that yellowing in the leaf of the main plant began on 22 October. It has been determined that the period of leaf yellowing of walnut genotypes continues for 31 days. When the walnut genotypes used in the study and the leaf yellowing dates of the main plant were compared, it was determined that the leaf yellowing period of all 80 genotypes started later than the main plant **(Table 2). It has been determined that the defoliation of the main plant started on 19 November.** In this aspect, it has been determined that 2 genotypes out of 80 genotypes studied are the same as the main variety, 5 genotypes are before the main variety, and the remaining 73 genotypes begin to defoliation later than the main variety **(Table 1). In studies carried out on Maraş 18 cultivars in different regions, it was determined that the defoliation period was between 8 November and 1 December (Sütyemez and Kaşka, 2002). It is thought that the difference between this study we conducted and those made by other researchers is due to climatic factors.**

Table 2. Classification of some phenological characteristics of walnut genotypes according to the main plant

	Bud break date	Leafing date	Date of yellowing in the leaves	Date of defoliation
Female Parent	16 March	26 March	**22 October**	**19 November**
Same as the main variety	3 genotypes (3.75%)	5 genotypes (6.25%)	-	2 genotypes **(2.5%)**
Before the Female variety	1 genotype **(1.25%)**	1 genotype **(1.25%)**	-	**5** genotypes (6.25%)
After the Female variety	**76** genotypes **(95%)**	74 genotypes **(92.5%)**	**80** genotypes **(100%)**	**73** genotypes **(91.25%)**

The genetic difference between walnut genotypes used in the study was determined by ISSR markers. In the study, 12 ISSR markers were used and polymorphic bands were obtained from 6 ISSR markers. As a result of the study, a total of 29 bands were obtained, 26 of which were polymorphic and 3 of which were monomorphic. It was determined that the polymorphism information content (PIC) value of genotypes ranged from 21% to 98%, and the average PIC value was 63% (Table 3).

Akcan et al. (2008), characterizations of 62 Walnut genotypes with ISSR and SRAP primers were determined. From the seedlings of the Kaman 1 variety, 112 bands were obtained by using 15 ISSR primers, 67 of which were polymorphic. In the seedlings belonging to the Kaman-5 variety, 92 bands were obtained from 15 ISSR primers and 54 of these were determined to give polymorphic bands. In this study we conducted, a total of 29 bands were obtained from seedlings obtained from Maraş 18 seeds by using ISSR primer and 26 of them were determined to be polymorphic. It has been thought that this is most likely due to the different gene and primers used.

Table 3. Allele numbers and polymorphism information content (PIC) values of the ISSR primers used

	No	Primers	Nucleotide Sequencing	Allels	PIC
ISSR	1	ISSR1	CACACACACACAA	3	0.94
	2	ISSR3	CACACACACACAGG	0	0
	3	ISSR4	CACACACACACAGC	3	0.57
	4	ISSR5	CACACACACACAG	6	0.98
	5	ISSR6	CACACACACACACAGT	0	0
	6	ISSR7	ACACACACACACACACCG	4	0.66
	7	ISSR8	ACACACACACACACACACC	4	0.42
	8	ISSR9	ACACACACACACACACTG	0	0
	9	ISSR11	GAGAGAGAGAGAGAGATC	0	0
	10	ISSR12	GAGAGAGAGAGAGAGAGAC	6	0.21
	11	ISSR13	AGAGAGAGAGAGAGAGC	0	0
	12	ISSR15	ATATATATATATATAT	0	0

Determination of genetic difference status of genotypes was made according to Nei (1972). The dendogram of the walnut genotypes used in the study was scanned with 6 ISSR markers, and the data obtained were calculated in NTSYSpc ver 2.2 program according to the UPGMA method. According to the dendogram obtained, it was determined that genotypes divided 2 main groups (Fig. 1). In this study, it has been determined that the Genotype 2 genetically closest according to the main plant. As a result of genetic analysis, it was determined that the main plant (Maraş 18) and 13 genotypes cluster in very close group, 49 genotypes in close group, 5 genotypes in far group and 13 genotypes in very far group.

Genetic cluster analysis was obtained from the Structure 2.3.4 program by calculating the ideal K value within the range of genetic analysis. In this clustering, the green areas are accepted as the genotypes close to Maraş 18 and the red coloured areas as the genotypes far from Maraş 18. In the study, it was evaluated that both colour groups are genetically different from each other (Fig. 2).

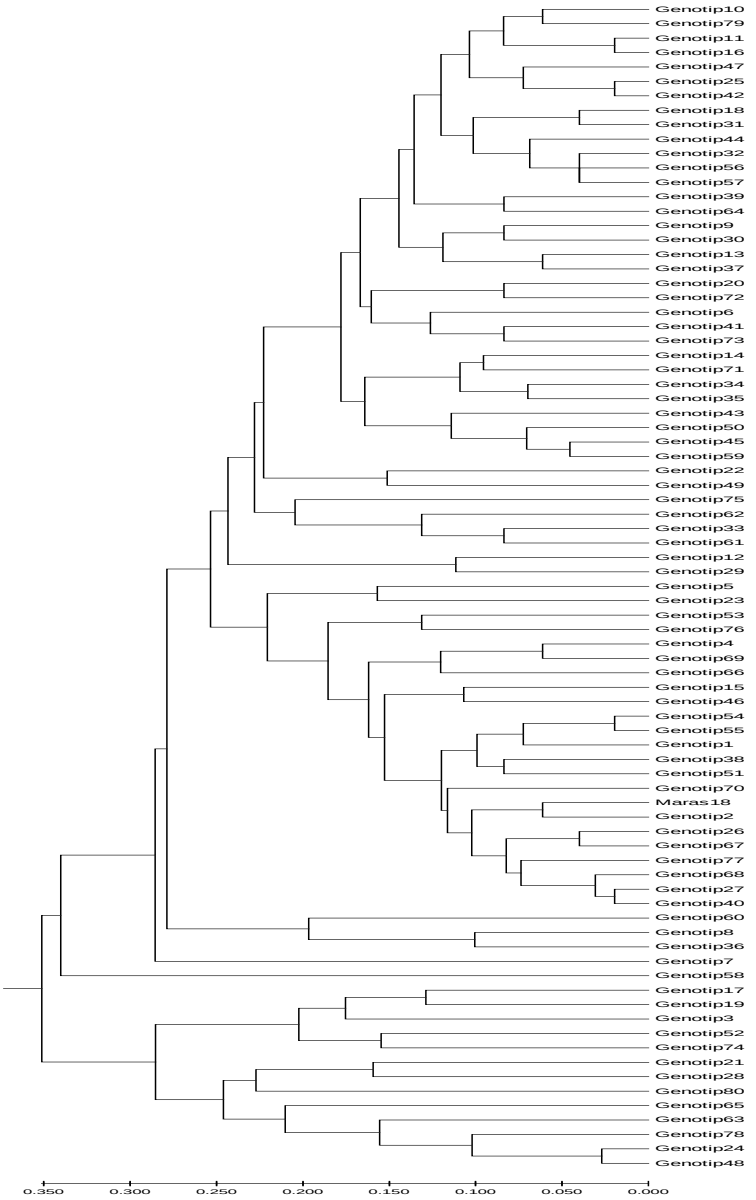


Figure 1. Dendrogram created according to the UPGMA method using Nei's genetic distance matrix

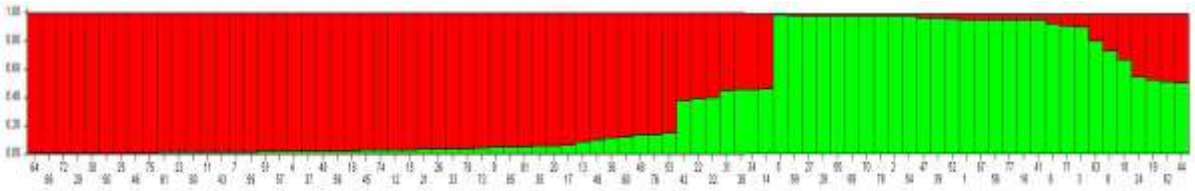


Figure 2. Cluster analysis of walnut genotypes with the Structure 2.3.4 program (ΔK=2)

Conclusions

In the research, it was determined that the genotypes of the seedlings obtained from the seeds of the Maraş 18 walnut varieties have a significant variation both phenologically and genetically. It has also been found that the diversity of parent plants and genotypes in terms of phenological characteristics is quite high. As a result of the research, it was confirmed that the

use of morphological and molecular data together in the management of gene resources provides a clear understanding of characterization. In addition, it has been determined that the ISSR marker technique can be used in the comparison of genetic variations and characterization of genotypes for walnut. This study is important in terms of revealing the phenological and genetic variation levels in the case of propagation from seed in the walnut, as well as providing resources for future breeding studies.

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INFLUENCE OF SUPERADSORBENT "TVERDAYA VODA" ON YIELD AND QUALITY OF POTATOES

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Abstract

The effect of the application of the superadsorbent "Tverdaya Voda" on the productive and qualitative properties of potatoes was investigated in experiments set up at two localities (East Sarajevo and Bijeljina). A control variant, superadsorbent "Tverdaya Voda", was used for these tests; superadsorbent "Tverdaya Voda" enriched with growth stimulants; superadsorbent "Tverdaya Voda" enriched with microorganisms; superadsorbent "Tverdaya Voda" enriched with microelements and superadsorbent "Tverdaya Voda" enriched with growth stimulants, microorganisms and microelements in the amount of 20 kg ha^{-1}. From the productive properties of potatoes were analyzed: number of tubers per plant, mass of tubers, yield, and from qualitative properties: dry matter content, mineral content and starch content. Compared to the multi-year averages, 2019 is characterized by higher temperatures and higher amounts of precipitation. The application of superadsorbent enriched with growth stimulants, microorganisms and microelements gave the highest number of tubers, while the highest mass of tubers and the highest yield was achieved by applying superadsorbent enriched with microorganisms. At the locality in East Sarajevo, a larger number of tubers per plant was determined, while at the locality in Bijeljina, tubers of higher mass were obtained and a higher yield of tubers per unit area was achieved. In the variant with adsorbent-enriched microorganisms, the tubers had the highest dry matter content and the highest starch content, while the highest mineral content was obtained by applying a superadsorbent enriched with microelements. In the control variant, the tubers had the lowest content of dry matter and starch. At the locality of East Sarajevo, the tubers had a higher content of dry matter and starch, while the content of mineral substances was higher at the locality of Bijeljina.

Keywords: *superadsorbent, potato yield, locality, tuber quality.*

Introduction

In Bosnia and Herzegovina, potatoes are grown on about 35,014 ha, with an average yield of 11.3 t ha^{-1} (Agency for Statistics of Bosnia and Herzegovina, 2019) and lags significantly behind potato yields in Europe and the world. Such low yields are mainly due to the use of poor quality planting material, poor agricultural techniques, production without irrigation, traditionally superficial attitude of producers towards production (Milic et al., 2012; Govedarica et al., 2015a: Govedarica et al., 2015b). In the conditions of climate change, the occurrence of more frequent droughts poses a threat to the sustainable production of agricultural crops, which can have a negative economic and sociological impact (Rivero et al., 2007). Water scarcity is one of the main causes of declining crop yields worldwide and a decrease in average yields of more than 50% in the most important field crops (Wang et al., 2003). Therefore, water scarcity, especially in arid and semiarid areas, is considered a major problem in food production (Zhang et al., 2014; Islam et al., 2011; Islam et al., 2011a; Zhao et

al., 2014). The application of natural and artificial substances that absorb water can be a viable alternative in solving the problem of lack and uneven distribution of precipitation. These substances can improve the physical and chemical properties of the soil, affect the content and availability of nutrients in the soil, have a positive effect on soil microorganisms and soil fertility (Mann et al., 2011). The introduction of hydrogels into sandy soils increases the germination and growth of plants, as well as the greater provision of soil with nutrients and water (Dorraji et al., 2010). Polymers can absorb moisture and fertilizers in the soil for up to five years after application before being broken down into non-toxic components (Holliman et al., 2005). By applying natural and synthetic improvers such as super-absorbent polymers, it is possible to provide good soil moisture in conditions of insufficient and unevenly distributed precipitation and thus enable the cultivation of plants in arid areas (Szczerski et al., 2013). The aim of this paper is to determine the influence of the superadorbent "Tverdaya Voda" (created at the Voronezh State Agrarian University named after Tsar Peter the Great) on the productive properties and quality of potatoes.

Material and methods

To examine the influence of "Tverdaya Voda" superadsorbents on yield, yield components, and potato tuber quality cultivars Agria, two-factorial experiments were performed: factor A different combinations of adsorbent and factor B - locality. Factor A consists of 6 variants: control variant (A_0); superadsorbent "Tverdaya Voda" (A_1); superadsorbent "Tverdaya Voda" enriched with growth stimulants (A_2); superadsorbent "Tverdaya Voda" enriched with microorganisms (A_3); superadsorbent "Tverdaya Voda" enriched with microelements (A_4) and superadsorbent "Tverdaya Voda" enriched with growth stimulants, microorganisms and microelements (A_5) in the amount of 20 kg ha-1. Factor B: locality B_1 - on the territory of the city of East Sarajevo, altitude 550 m (43 ° 49'01 " NW and 18 ° 20'57 " EWD) on alluvial soil (fluvisol) and locality B_2 - on the territory of the city of Bijeljina, altitude 90 m (44 ° 41 ′ N; 19 ° 14 ′ E) on semi-glacial land. The experiments were set up according to a randomized block system in four replications with a set of 53,333 plants per ha^{-1}. The area of the basic plot is 15 m2 (four rows 5 m long with 20 plants in a row, with a distance between rows of 0.75 m and between plants in a row of 0.25 m). The common agrotechnical measures have been applied in potato production. Primary treatment, preparation and soil fertilization were performed in a way standard crop of potatoes calls for. The protection of crops from weeds, pests and disease-causing agents were conducted in a timely manner. Of the productive traits, the following were monitored: the number of tubers per plant; the average mass of the tuber and the yield of the tuber calculated per hectare (t ha^{-1}). The experiment in Bijeljina was set on March 24, 2019. Potatoes began to germinate evenly on April 13, 2019, and bloom on June 6, 2019. Potato harvestwas done on August 24, 2019. The experiment in East Sarajevo was established in April 25, 2019. Potatoes began to germinate evenly on May 15, 2019, and bloom on June 29, 2019. Potato harvest was done on September 14, 2019. The chemical analysis of the tuber includes the following properties: dry matter content in the tuber (%), by drying the plant material in oven at a temperature of 105°C to a constant mass; mineral content in the tuber (%), by annealing the plant material at a temperature of 550°C to constant weight; the starch content in the tuber (%) was determined polarimetrically according to *Ewers* (1908). The obtained results were processed by the method of descriptive statistics. Examination of the significance of differences between treatments was performed by analysis of variance (ANOVA). Significance of differences was tested by Fisher's LSD test. Statistical processing was done using the statistical program STATISTICA 10 (StatSoft, Inc. Corporation, Tulsa, OK, USA). In order to see the basic indicators of weather conditions during the survey, meteorological data of average monthly temperatures and precipitation

amounts for 2019 were used, taken from the Hydrometeorological Institute of the Republic of Srpska and the Federal Hydrometeorological Institute of Bosnia and Herzegovina, from measuring stations closest to field surveys. The values of these meteorological elements, as well as multi-year averages are shown in Table 1. The average annual temperature for East Sarajevo and the surrounding area in 2019 was 11.7°C and was higher than the lon-term average, while the total rainfall was 850.5 mm, which is less than the long-term average (932 mm). The average annual temperature for Bijeljina and its surroundings in 2019 was 13.6°C and was higher than long-term average of 11.5°C, while the total rainfall was 839.4 mm, which is less than long-term average (778,2 mm). Compared to long-term averages, 2019 is characterized by higher temperatures and an uneven distribution of precipitation. Only in May, temperatures were lower at both sites, which significantly affected the slower emergence of potatoes in East Sarajevo. In July and August, a deficit of precipitation was noted in Bijeljina, and in East Sarajevo, the values determined in these months were on the verge of a deficit.

Table 1. Average monthly air temperatures (°C) and precipitation (mm) during 2019 and long-term average

Month / Year			I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Average/ sum
E. Sarajevo	2019	°C	-1,5	2,5	7,7	11,4	12,3	21,1	21	21	16,5	12,9	10,6	3,6	11,7
		Mm	79,7	56,7	51,8	98,2	102,3	97,1	67,8	69,7	44,1	38	80,3	64,8	850,5
	1981-2010	°C	-0,1	1,4	5,3	9,9	15,0	17,7	19,8	19,7	15,1	10,9	5,2	1,1	10,1
		Mm	67	63	71	78	73	94	72	70	86	85	91	85	932
Bijeljina	2019	°C	0,6	4,7	10,2	13,2	14,9	23,7	23,2	24,3	18,1	13,7	11,3	4,8	13,6
		Mm	62,8	34,1	33,7	95,4	121	113	59,2	27	57,2	27,6	52,7	68,1	751,8
	1981-2010	°C	0,3	1,9	6,9	11,8	16,9	19,9	22,1	21,2	17,2	11,6	6,0	2,3	11,5
		Mm	54,1	43,5	59,7	66,1	68,2	100,0	74,6	60,7	55,8	67,6	64,0	63,9	778,2

Results and discussion

Table 2. shows the influence of adsorbent application and localities on the productive properties and quality of potatoes. The application of different variants of the adsorbent had a highly significant effect on the examined properties, while the influence of the locality had high significance for all tested properties except for the average weight of the tuber. Under the influence of the adsorbent x locality interaction, significant variations in tuber mass, potato yield and mineral content in tubers were found.

Table 2. Influence of adsorbent and locality on productive and qualitative properties of potatoes

Factor		Number of tubers	Mass of tubers	Yield	% dry matter	% minimal matter	% starch
Adsorbent (A)		**	**	**	**	**	**
Locality (B)		**	nssd	**	**	**	**
A * B		nssd	*	*	nssd	*	nssd
A	$LSD_{0.05}$	0,314	4,779	1,671	0,642	0,143	0,642
	$LSD_{0.01}$	0,422	6,420	2,244	0,863	0,192	0,863
B	$LSD_{0.05}$	0,181	2,759	0,965	0,371	0,082	0,371
	$LSD_{0.01}$	0,243	3,707	1,296	0,498	0,111	0,498
A*B	$LSD_{0.05}$	0,444	6,758	2,363	0,909	0,203	0,909
	$LSD_{0.01}$	0,597	9,079	3,174	1,221	0,272	1,221

(*) statistically significant difference, (**) statistically very significant difference, (nssd) there is no statistically significant difference

Many authors point out that the number of tubers per plant is a varietal trait, but largely depends linearly on the number of primary (main) trees per plant, seed tuber size, agroecological conditions and production technology (Tadesse et al., 2001; Gvozden, 2016; Momirovic et al., 2016). Barkley (2005) and Gvozden (2016) in their research obtained a higher number of tubers per plant at lower than at higher air temperatures. Ierna and Parisi (2014) state in their research a significant influence of cultivation methods on the number of tubers per plant and the average weight of tubers. The average number of tubers per potato plant regardless of the application of adsorbent and locality is 8.3 (Table 3). The control variant had the lowest number of tubers per plant (7.8), and the highest superadsorbent enriched with growth stimulants, microorganisms and microelements (9.0). The determined differences are statistically highly significant, as well as the differences of other variants of superadsorbent in comparison with the control variant. The number of tubers per plant in Bijeljina is 8.2, and in East Sarajevo 8.5. These differences were highly significant.
The average weight of the tuber is a varietal characteristic, but it largely depends on the action of agroecological factors, applied agrotechnics, the way of forming the house (nest), the size of the seed tuber, the number of trees per plant, the number of tubers per plant, the length of stolons (Gvozden, 2016). Higher temperatures stimulate vegetative development, reduce tuber formation and average tuber mass, tuber yield, harvest index and dry matter concentration in tubers (Tadesse et al., 2001). The average weight of potato tubers regardless of the application of adsorbent and locality is 98.6 g (Table 3).

Table 3. Average number of tubers, average weight and yield of potatoes depending on the adsorbent in selected localities

Adsorbent / Locality	A_0	A_1	A_2	A_3	A_4	A_5	Average
Number of tubers per plant							
E. Sarajevo (B_1)	7,6	8,0	8,1	8,1	8,3	8,8	8,2 b
Bijeljina (B_2)	8,0	8,4	8,4	8,4	8,6	9,1	8,5 a
Average	7,8 c	8,2 b	8,3 b	8,3 b	8,5 b	9,0 a	8,3
Average mass of tubers (g)							
E. Sarajevo (B_1)	94,8 d	96,0 d	100,4 bcd	106,1 ab	95,1 d	97,8 cd	98,4
Bijeljina (B_2)	85,3 e	100,0 bcd	104,5 bc	111,1 a	95,3	97,0 d	98,9
Average	90,0 d	98,0 bc	102,4 b	108,6 a	95,2 c	97,4 c	98,6
The yield of potatoes (t ha^{-1})							
E. Sarajevo (B_1)	38,5 g	41,1 f	43,3 def	45,7 bc	42,1 ef	45,8 bc	42,7 b
Bijeljina (B_2)	36,2 g	44,6 cd	47,0 b	49,9 a	43,9 cdf	47,1 b	44,8 a
Average	37,3 e	42,8 d	45,1 c	47,8 a	43,0 d	46,4 b	43,8

Adsorbent: Control variant (A_0); superadsorbent "Hard water" (A_1); superadsorbent "Hard water" enriched with growth stimulants (A_2); superadsorbent "Hard water" enriched with microorganisms (A_3); superadsorbent "Hard water" enriched with microelements (A_4) and superadsorbent "Hard water" enriched with growth stimulants, microorganisms and microelements (A_5).

The mass of the tuber ranged from 90.0 grams to 108.6 grams. Using superadsorbent enriched with microorganisms (108.6 g), potato tubers were obtained that had a statistically significantly higher mass of tubers compared to other variants, while compared to variant A_2 (adsorbent enriched with growth stimulants), this difference was significant. The control variant had a statistically highly significant lowest tuber weight compared to the application of different variants of superadsorbent, except for variant A_4 (superadsorbent enriched with microelements) where the identified differences were significant. Highly significant variations in tuber mass between superadsorbent enriched with growth stimulants (A_2) and superadsorbent enriched with microelements (A_4) were also found, while differences between

variant A_2 and A_5 were significant. The average weight of tubers in Bijeljina is 98.9 g, and in East Sarajevo 98.4 g. The differences found did not matter. At both localities, the highest average mass of tubers was in variant A_3, and the lowest in the control variant. At the Bijeljina locality, the average tuber mass was higher in variants A_1, A_2, A_3 and A_4, while at the East Sarajevo locality the average tuber mass was higher in the control variant and variant A_5. The adsorbent x site interaction had a significant effect on the average tuber mass.

Potato yield depends on the variety and its genetic potential, agroecological conditions and levels of applied agrotechnics, seed tuber size, number of trees per plant and number of tubers (Khan et al., 2004; Momirovic et al., 2016; Gvozden, 2016; Postic et al., 2015). The average yield of potato tubers regardless of the application of adsorbent and locality is 43.8 t ha^{-1} (Table 3). Tuberous yields ranged from 37.3 t ha^{-1} to 47.8 t ha-1. The application of superadsorbent enriched with microorganisms (37.3 t ha^{-1}) resulted in a statistically highly higher yield of tubers in comparison with other variants, while in comparison with variant A_5 the determined difference did not matter. Different variants of the applied adsorbent achieved a highly significantly higher yield of tubers compared to the control variant. Highly significant and significant variations between the examined adsorbent variants (between variant A1 and variants A_2 and A_5; between variant A_3 and variants A_2 and A_5) were also found. These results are consistent with the results of Lukina et al. (2019) who in two-year experiments obtained higher yields on plots on which they applied 20 kg of ha^{-1} superadsorbent enriched with microorganisms, where, depending on the year, the increase in potato yield ranged from 1.1 to 1.5 times. The average tuber yield in Bijeljina is 44.8 t ha^{-1}, and in East Sarajevo 42.7 t ha^{-1}. The differences found were highly significant. At the locality of East Sarajevo, the highest tuber yield was in variant A_5, and the lowest in the control variant, while at the locality in Bijeljina, the highest tuber yield was achieved in variant A_3, and the lowest in the control variant. When comparing the yield between the examined localities, a higher yield was found in the variants with the adsorbent at the locality in Bijeljina, while a higher yield in the control variant was determined in East Sarajevo.

The importance of water retention in the soil is always extremely important for regions with insufficient rainfall because it inevitably affects production capacity (Rode, 2008). Agricultural production in arid areas faces many problems, especially when the lack of rainfall coincides with a particular vegetation phase of plants (Kadyrov et al., 2005). Therefore, the solution to such problems should be aimed at developing efficient water-saving technology and thus efficiently providing sufficient moisture to plants. The use of adsorbents with the addition of microorganisms in order to retain moisture in the soil contributes to environmental safety in crop production. Providing plants with the necessary moisture during a certain vegetation period as well as increasing microbiological activity in the root area will definitely reduce the effect of stress from adverse weather conditions and increase production capacity (Lukin et al., 2019).

The dry matter content in both tubers and aboveground parts of the plant is significantly influenced by environmental conditions and agrotechnical measures (Geremew et al., 2007). The average dry matter content in potato tubers, regardless of the application of adsorbent and locality, is 25.2% (Table 4). The control variant had the lowest dry matter content in potato tubers (24.3%), and the highest superadsorbent enriched with microorganisms (26.0%). The determined differences are statistically highly significant, as well as the differences of other variants of superadsorbent in comparison with the control variant, except for variant A_2 where the determined differences did not have statistical significance. The average dry matter content in potato tubers in East Sarajevo (25.7%) is highly significantly higher compared to Bijeljina (24.8%).

The average mineral content in potato tubers regardless of the application of adsorbent and locality is 1.3% (Table 4). The content of mineral substances in the tuber ranged from 1.1% to

1.6%. The application of different variants of superadsorbent highly significantly influenced the content of mineral substances in the tuber. A highly significant influence of the locality was also determined, while the adsorbent x locality interaction significantly influenced the mineral content in the tuber. In variants A1 and A4, a significantly higher content of minerals in the tuber was found, and in comparison with other examined variants.

Table 4. Average content of dry matter, minerals and starch in potato tubers depending on the adsorbent in selected localities

Adsorbent / Locality	A_0	A_1	A_2	A_3	A_4	A_5	Average
Content of dry matter (%)							
E. Sarajevo (B_1)	24,8	25,8	25,2	26,5	26,0	25,8	25,7 a
Bijeljina (B_2)	23,9	24,9	24,3	25,6	25,0	24,9	24,8 b
Average	24,3 d	25,3 bc	24,7 c	26,0 a	25,5 ab	25,3 bc	25,2
Content of mineral matter (%)							
E. Sarajevo (B_1)	1,338 cd	1,560 ab	1,430 abc	1,383 bc	1,613 a	1,345 cd	1,445 a
Bijeljina (B_2)	1,148de	1,555 ab	0,958 e	1,123 e	1,448 abc	0,945 e	1,196 b
Average	1,243 b	1,558 a	1,194 b	1,253 b	1,530 a	1,145 b	1,320
Content of starch (%)							
E. Sarajevo (B_1)	17,5	18,5	17,9	19,2	18,7	18,5	18,4 a
Bijeljina (B_2)	15,0	16,0	15,43	16,7	16,2	16,0	15,9 b
Average	16,3 d	17,3 bc	16,7 d	18,0 a	17,4 ab	17,3 bc	17,2

Adsorbent: Control variant (A_0); superadsorbent "Hard water" (A_1); superadsorbent "Hard water" enriched with growth stimulants (A_2); superadsorbent "Hard water" enriched with microorganisms (A_3); superadsorbent "Hard water" enriched with microelements (A_4) and superadsorbent "Hard water" enriched with growth stimulants, microorganisms and microelements (A_5)

The average mineral content in potato tubers in East Sarajevo (1.445%) was highly significantly higher compared to Bijeljina (1.196%). At the locality Istočno Sarajevo, the highest content of mineral substances in potato tubers was in variant A_4, and the lowest in the control variant, while at the locality in Bijeljina, the highest content of minerals in potato tubers was achieved in variant A_1, and the lowest in variant A_5. During the analysis of the interaction of the adsorbent x locality, a significantly higher content of mineral substances in the tuber was determined for all variants of adsorbents at the locality in East Sarajevo, except for variant A_1.

The starch content in tubers is influenced by the method of cultivation and genotype (Geremew et al., 2007; Tein et al., 2014; Brocic et al., 2016), where late varieties stand out due to longer vegetation period and longer period of accumulation of photosynthetic substances. (Singh and Lovedeep, 2009). The average starch content in potato tubers regardless of the application of adsorbent and locality is 17.1% (Table 4). The control variant had the lowest starch content in potato tubers (16.3%), and the highest superadsorbent enriched with microorganisms (18.0%). The determined differences are statistically highly significant, as well as the differences of other variants of superadsorbent in comparison with the control variant, except for variant A_2 where the determined differences did not have statistical significance. The average starch content in potato tubers in East Sarajevo (18.4%) is highly significantly higher compared to Bijeljina (15.9%), which is in accordance with the results of Tein et al. (2014) who state that larger tubers have a lower starch content, compared to smaller tubers in general.

Conclusion

The application of superadsorbent enriched with growth stimulants, microorganisms and microelements (A_5) gave the highest number of tubers, while the highest mass of tubers and the highest yield was achieved by applying superadsorbent enriched with microorganisms (A_3). Larger tubers and high yield per unit area were achieved by applying superadsorbent enriched with growth stimulants (A_2) and superadsorbent enriched with growth stimulants, microorganisms and microelements (A_5). In the variant with adsorbent-enriched microorganisms, the tubers had the highest dry matter content and the highest starch content, and in the application of superadsorbent enriched with microelements, the tubers had the highest mineral content, and in the control variant the lowest dry matter and starch content. In the variant with superadsorbent enriched with growth stimulants, microorganisms and microelements, the tubers had the lowest ash content.

At the locality in East Sarajevo, a larger number of tubers per plant was determined, while at the locality in Bijeljina, tubers of higher mass were obtained and a higher yield of tubers per unit area was achieved. At the locality in East Sarajevo, the tubers had a higher content of dry matter, the highest content of starch, while the content of mineral substances was higher at the locality of Bijeljina.

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EFFCT OF NATURAL ZEOLIT AND DROUGHT STRESS ON SOME PHYSIOLOGICAL AND MORPHOLOGICAL TRAITS OF *FESTUCA ARUNDINACEA* GRASS

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Abstract

Grass is one of the most important components of landscape in the world. Zeolites due to their high CEC can play a nutritional role in addition to soil modification and improve plant growth especially in low cation exchange lands and in plants under drought stress. So, this study was designed to investigate the effect of zeolite and drought stress on physiological and morphological characteristics of *Festuca arundinacea* grass. A factorial experiment based on completely randomized design with 2 treatments and 3 replications was conducted in horticultural science greenhouses of Ferdowsi University of Mashhad, Iran, in 2019. Treatments included 3 levels of drought stress based on field capacity (FC): (100% (control), 50% and 25%) and 3 levels of zeolite mixed with loam: (0, 10% and 20% w / w). According to the results, drought levels resulted in reduced morphological traits, decreased RWC and increased EL, proline and total carbohydrate content in the plant. The interaction effects of zeolite and drought stress showed that treatment with 20% zeolite had the highest leaf area (31640 cm ^{2}), total dry weight (78.21 g) and carotenoids (2.16 mg / g fresh weight) and the lowest EL in non-stress conditions. Generally, drought levels of 50 and 25% increased the amount of carbohydrates and proline. Application of zeolites, especially at 20% level, increased morphological traits such as root and shoots dry weight, leaf area, height and carotenoids. In general, the application of zeolite has led to increased growth traits in grass under drought stress.

Keywords: *Carbohydrate, Chlorophyll, Ion leakage, Proline*

Introduction

Water is one of the most limited factors in agricultural production worldwide (Sankar et al., 2007). Climate change in the last decade has reduced rainfall in the world, especially in the Middle East (Alfi and azizi, 2015). Plant physiological traits such as leaf growth, stomatal conductance, photosynthetic rate and nitrogen metabolism are reduced during drought stress. Under drought stress the plant water potential decreases. Decreased water potential reduces cell growth and protein synthesis. Carbon dioxide flow and leaf transpiration are reduced and proline and abscisic acid accumulation is increased (Heidaiy and Moaveni, 2009). On the other hand, soil is one of the most important environmental factors and as the main source in providing essential plant elements and water (Ghaemi et al., 2014). Due to the limited water resources in Iran and especially the recent droughts, maintaining soil moisture in various ways is one of the proposed ways to prevent the effects of drought stress (Gholizadeh, 2005). Soil texture is an important non-biological factor in the distribution of minerals and the preservation of soil organic matter and microbial mass. Pore distribution has an important effect on the abundance of fungi and bacteria, which itself plays an important role in carbon mineralization (Hamarashid et al., 2010). Today, one of the new and effective methods to improve the substrate and preserve water and minerals in the soil is the use of zeolites. Global

production of natural zeolite in 2013 is estimated at 2.7 to 3.2 million tons (Virta, 2013). The high ability of zeolites to absorb and store water allows the water used by the plant to be stored and used when necessary. Due to their high cation exchange capacity and the presence of some cations, including ammonium in their network, zeolites can play a nutritional role in addition to soil remediation and improve plant growth, especially in areas with low cation exchange capacity (Harb and Mahmoud, 2009). It has been reported that natural zeolite may be an important alternative to reduce the effects of drought in arid and semi-arid regions (Ghanbari and Ariafar, 2013). Zeolite and water stress have been reported to have a significant effect on most of the measured growth parameters (Gholi zade et al., 2004). According to research, the use of zeolite can be a way to improve the soil, increase the effects of chemical and organic fertilizers and as an underlying component for the development of various crops (Najafi-Ghiri, 2014). Due to the expansion of cities and the mechanization of modern life today, the importance of green spaces has become more and more apparent and has been considered. Green space can significantly moderate the air temperature and help to soften the air (Shiravand, 2011). One of the most important components of green space are cover plants and grass is one of the most important cover plants in the world (Hosseini et al., 2015). In fact, no plant like grass can provide the beauty and freshness of a green environment (Tehranifar et al., 2009). Grass plays the most important role in air purification and reducing its pollution in urban environments (Shiravand, 2011). The genus Festuca includes more than 360 species that differ greatly in appearance. Less than ten species are used as meadows. *Festuca arundinacea* grass is a perennial cover plant with deep roots and is suitable for the cold season. Considering the importance of drought stress in green space and the effect of zeolite on water retention, this study was designed and conducted to investigate the effect of zeolite and drought stress on physiological and morphological characteristics of *Festuca arundinacea* grass.

Materials and methods

In order to investigate the effect of zeolite on quantitative and qualitative traits of grass plants under drought stress, a factorial experiment based on a completely randomized design with 2 treatments and 3 replications was conducted in the greenhouses of horticulture at the Faculty of Agriculture, Ferdowsi University of Mashhad, Iran in 2019. The tested treatments included irrigation levels at three levels (100% (control), 50% and 25% of field capacity) and the amount of zeolite mixed with loamy soil at three levels (0, 10 and 20% w/w). The measurement of traits began one month after the drought stress and after observing the signs of stress on the grass. Leaf area was measured with a leaf area meter. Plant height, shoot and root dry weight were measured. The relative water content was calculated after observing the symptoms of stress and in according to Omae et al. (2007)

To measure electrolyte leakage, 2 cm leaf pieces were placed in test tubes after washing with 10 ml of distilled water. The tubes were then shaken vigorously for 17 to 18 hours. Then (Ci) was measured by a conductometer (JEN WAY model 4310). The test tubes were transferred to an autoclave at a temperature of 121 ° C and a pressure of 1.2 atmospheres for 15 minutes. And (Cm) was measured. Then the electrolyte leakage values were calculated according to the following equation:

$EL = (Ci / Cm) \times 100$

Carotenoid measurements was used by Dere (1988) method. To measure proline and soluble carbohydrates, extraction was performed, and to measure proline, the samples were transferred to a spectrophotometer and then absorbed at 520 nm (Bates, 1973). The amount of soluble sugars was measured by Irigoyen (1992) method.

Data analysis: Data analysis was performed by jmp 8 software. Graphs were drawn using Excel and the means were compared with LSD test at the level of 5% error probability.

Results and discussion

Root and shoot dry weight, plant height, leaf area: According to the results of table 2, drought stress led to a decrease in grass dry weight. So that by increasing the stress intensity from 100 to 25% of field capacity, shoot dry weight, root dry weight and total dry weight were decreased by 28.49, 32.50 and 32.17% respectively.

Table 1. Simple effect of drought stress and soil texture on quantitative and qualitative traits measured in *Festuca arundinacea*

Treatments		Plant height (cm)	Relative water content (%)	Proline (mg/gfw)	carbohydrate (µg/gfw)
Drought stress (FC%)	100	18.50a	80.17a	0.96b	0.50b
	50	17.22b	77.99a	1.66a	0.87a
	25	14.44c	50.04b	0.99b	0.92a
Zeolite (%)	0	17.56a	61.12b	1.50a	0.80a
	10	16.56ab	74.56a	1.35a	0.71a
	20	16.06b	72.52a	1.50a	0.78a

Means that have common alphabetic in each trait do not significant difference at level %1 base on LSD test

Table 2. Interaction effects of drought stress and soil texture on quantitative and qualitative traits measured in *Festuca arundinacea*

Drought stress (FC%)	Zeolite (%)	Root dry weight (g/plant)	Shoot dry weight (g/plant)	Total dry weight (g/plant)	Leaf area (cm^2)	Electrolyte leakage (%)	Carotenoids (mg/gfw)
100	0	16.67de	7.93de	24.60de	9952d	25.25bc	0.75e
	10	37.73b	17.97b	55.70b	22530b	18.69def	1.89abc
	20	52.98a	25.23a	78.21a	31640a	13.85f	2.13a
50	0	15.00e	7.14e	22.14e	8957de	30.59ab	1.39d
	10	25.33c	12.06c	37.40c	15130c	19.59cdef	1.73c
	20	34.10b	16.24b	50.33b	21030b	20.53cde	2.16a
25	0	11.25e	5.67e	16.93e	7121e	36.11a	0.82e
	10	21.53cd	10.25cd	31.78cd	12860c	23.98cd	1.84bc
	20	33.82b	16.89b	50.71b	22180b	14.75ef	2.11ab
LSD		6.23	2.58	8.75	2762	6.02	0.28

Means that have common alphabetic in each trait do not significant difference at level %1 base on LSD test

However, the use of zeolite mixed with potting soil led to an increase in plant dry weight, so that the maximum shoot dry weight, root dry weight and total grass dry weightwere in the treatment of 100% FC + 20% zeolite at the rate of 25.23, 52.98 and 78.21 g per plant, respectively. With increasing drought stress from 100 to 25% of field capacity, grass height decreased by 4 cm (Table 1). The highest leaf area (31640 cm2) was in 100% FC + 20% zeolite and the lowest value was in non-use of zeolite, and in 25% FC (7121 square centimeters) and 50% FC (8975 square centimeters). Plant growth is done by evaluating the effect of treatments on traits such as root length, height, roots and shoots dry weight and leaf area. In this study, drought stress at 50 and 25% FC led to a decrease in vegetative traits such as plant height. It can be said that drought stress leads to an imbalance between antioxidant defense and the reactivity of reactive oxygen species (ROS) and thus oxidative stress. ROS is essential for intracellular signaling but in high concentrations can damage various cell surfaces including chloroplasts. ROS has the ability to initiate lipid peroxidation and degrade

proteins, lipids, and nucleic acids (Hendry, 2005). On the other hand, leaf mesophilic cells become dehydrated due to drought, abscisic acid is stored in the protective cells used in chloroplasts, and ABA production is increased in protective cells and mesophilic cells. As ABA increases, potassium and calcium are released from the protective cell. The result of this pore closure process is the loss of water in the protective cell, and with water deficiency, the rate of photosynthesis in plants decreases and consequently plant growth decreases (Fathi et al., 2016).

Ion leakage, relative content of leaf water, carotenoids: The results showed that irrigation levels of 100 and 50% of FC without significant difference had higher relative water content (80 and 78%, respectively) 25% FC (50% of relative water content) (Table 1). Also, both 10 and 20% zeolite levels increased the relative leaf water content without significant differences. Examination of ion leakage results in Table 2 showed that reducing the amount of irrigation water has led to an increase in ionic leakage in grass leaf cells. So that with increasing the stress intensity from 100 to 25% of field capacity, the amount of ion leakage increased from 25% to 36%. Both levels of zeolite used in this experiment were effective in reducing ion leakage under stress conditions. So that in drought stress (25%), with the application of zeolite in two levels of 10 and 20% by weight, the amount of ion leakage decreased from 36.1% in the absence of zeolite to 23.9 and 14.7%. Relative water content is one of the important physiological parameters in the study of plant resistance to stress and plants that have more tolerance to drought stress have higher relative water content (Paknejad et al., 2007). According to the above results on grass, the use of zeolite has a significant effect on increasing the relative water content. Zeolites cause horizontal distribution of water in the soil and reduce the effects of drought stress on the relative water content of the plant by increasing the water holding capacity in the soil (Polat et al., 2004). The interaction effect of zeolite and drought stress on the carotenoids in grass leaves showed the lowest amount of carotenoids in two treatments of 25 and 100% of field capacity and in the absence of zeolite. According to the results of Table 2, with increasing stress from 100 to 50 and 25% of field capacity, the carotenoids in the treated grasses increased from 0.75 to 1.39 and 0.82 mg / gr. Drought stress has been reported to increase carotenoid levels by decreasing chlorophyll synthesis and increasing the accumulation of secondary metabolites, including anthocyanins (Arazmjio et al., 2010).

Total soluble proline and carbohydrate content: With decreasing irrigation water, total carbohydrate in the plant increased. So that the amount of carbohydrates in the absence of drought stress was 0.50 µg and at 50 and 25% FC reached 0.87 and 0.92 µg/gr. According to the results of this study, the amount of carbohydrates and proline was affected by drought stress and irrigation levels of 50 and 25% FC had higher levels of proline and carbohydrates than 100% FC. Changes in physiological processes are one of the most important events when the plant is exposed to drought stress (Liu et al., 2011). On the other hand, under osmotic stress, the amount of soluble sugars in the cell increases. This phenomenon is probably the adaptive mechanism of the plant to maintain the osmotic potential under osmotic stress. In addition, with this mechanism, the plant can maintain its carbohydrate storage under stress conditions to maintain optimal basal metabolism (Verma and Dubey, 2001).

Conclusion

According to the results of this study, relative water content and the amount of ion leakage had a significant increase in 25% FC compared to the other two levels. Drought stress increased carbohydrate and proline levels. The use of zeolites, especially at the level of 20%, led to an increase in growth traits such as dry weight of roots and shoots, leaf area, height and

carotenoids. In general, the application of zeolite has led to an increase in growth traits in grasses under drought stress and it can improve effects on plant growth.

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INVESTIGATION OF PHYSICOCHEMICAL PROPERTIES OF SOME PLUM CULTIVARS OF IRAN

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Abstract

Plums have been known as one of the most diverse fruits in temperate regions, although their variation and diversity also cause differences in the physicochemical features of the fruit. Plums, on the other hand, owing to their high moisture content and short harvest season, are mostly and have been consumed. Therefore, the knowledge of characteristics of the cultivars and consider choosing the best drying conditions for drying in order to maintain the quality and nutritive value. The present study was conducted to investigate the physicochemical properties of 7 Iranian plum cultivars in a randomized complete block design with 5 replications. Plum cultivars with the usual names included: Ghatretala, Pivehzhan, Rotaby, Ghandy, Beygom, Torghabeh Sabz and Bokhara. Analysis of variance demonstrated that all cultivars had significant differences ($p < 0.01$) for all measurement indexes of chemical and physical features (except for kernel thickness). The results also showed that in most of the chemical features studied, cultivars including Beygom and Torghabeh Sabz were superior to other cultivars and had the highest amount of chemical compounds among the cultivars. The results also showed that there was a high amount of compounds such as antioxidants, flavonoids and vitamin C in the cultivars. Therefore, it can be said that some of the cultivars investigated in this study were rich in chemical combinations and had high nutritive values.

Keywords: *Antioxidant Capacity, dried plum, kernel thickness, Vitamin C*

Introduction

Plums belong to the family Rosaceae, subfamily Pronoideae and genus *Prunus*. Plum is one of the most diverse fruits that grows in a wide range of climatic and geographical conditions. This product is native to Europe and Asia (Karimifar et al., 2012). Plums contain carbohydrates (sucrose, glucose, fructose), organic acids (citric and malic acid), fibers, tannins and aromatic compounds that determine the nutritional value and taste of plums (Arion et al., 2014). It is also a good source of carotene, anthocyanins and natural antioxidants (Kim et al., 2003). Jalili et al. (2011) conducted an experiment on 38 genotypes of Iranian plums and reported that the soluble solids content in plum genotypes is in the range of 16 to 31 degrees Brix. Davarinejad et al. (2010) also studied 9 apricot cultivars and reported the amount of soluble solids content of the fruit is in the range of 15.40 - 9.43 degrees Brix. The amount of titratable acid in different plum genotypes varied from 0.4 to 0.94 (20). According to Vangdal (1980) studies, the acceptance of plums with a solids content of less than 12 degrees Brix is very low for most consumers. The better taste of plum fruit and its greater acceptance depend on less titrable acidity and higher soluble solids content (% 12%) of the fruit.

Material and Methods

Plant material

This research was conducted in Ferdowsi University of Mashhad in 2019. Seven important plum cultivars from one of the commercial orchard of Neishabour city in Khorasan Razavi province were picked at full maturity stage and transferred to the laboratory. The experimental design was a randomized complete block design (RCBD) with 7 treatments (plum cultivars) and five replications. Due to the fact that the maturity of different cultivars was happened on different days, the cultivars were collected over a period of four months.

Physical properties

The weight of the fresh fruit and its kernel were determined using a digital laboratory balance (accuracy: 0.001 g). The three linear dimensions of length (L), width (W) and thickness (T) were measured with a digital caliper (accuracy: 0.01 mm). The firmness of fruit texture was used by penetrometer (8 *mm* Tip) and recorded in N/cm^2 (Davarinejad et al., 2010).

Biochemical properties

The soluble solid content of fruit juice was immediately measured using a digital refractometer. The titrable acidity was determined based on mg of malic acid (major organic acid) in 100 g of fruit tissue by titration of 10 ml of fruit extract with 0.1 N sodium hydroxide solution to reach pH = 8.3 (Najafzadeh et al., 2012). Finally, the flavor index was obtained based on the TSS / TA ratio (Davarinejad et al., 2010). The ascorbic acid was measured by titration method and expressed as mg in 100 ml of fruit juice (AOAC, 2005). The antioxidant capacity of plum fruits was determined by the free radical scavenging properties of DPPH. Finally, the antioxidant capacity of fruit juices was reported as a percentage of inhibition (DPPHsc %). Total flavonoids of fruits were measured according to Gil et al. (2002) at 506 nm.

Statistical analysis

Statistical analysis was performed using JMP 9 statistical software (SAS, Institute, and Cary, NC). The standard error in the tables was also shown as (SE ± mean).

Results and discussion

The results of analysis of variance showed a significant difference between the plum cultivars in terms of physical characteristics including fruit and kernel dimensions ($p \leq 0.01$). The results also showed that plum cultivars have significant effects on the soluble solid content (Brix), titrable acidity, flavor index, vitamin C, antioxidant capacity and flavonoids.

According to the results of fruit dimensions, Pivehzhan cultivar had the highest values of fruit length and width. While Ghandy and Beygum cultivars had the lowest length and width of fruit, respectively. The maximum and minimum forces required for firmness of fruit tissue with values of 2.19 and 2.02 N/cm2 were observed in Beygum and Torqhabeh Sabz cultivars, respectively. Previously, the average stiffness in four plum cultivars has been reported by Yousefi and emam-djomeh (2015) as 1.87 N/cm^2. Calisir et al., (2005) also studied the physical properties of some wild plums and determined the firmness values of fruit tissue between 0.625-0.499 N/cm^2.

Table 1- Physical properties of plum cultivars

Cultivar	Fruit length (mm)	Fruit width (mm)	Fruit weight (g)	Kernel weight (g)	Firmness (N/cm^2)
Ghatretala	34.19±0.3	34.59±0.3	24.52±0.6	1.35±0.1	1.67±0.06
Pivehzhan	36.55±0.2	34.71±0.3	26.24±0.4	1.54±0.3	1.30±0.1
Rotaby	35.08±0.3	30.36±0.2	21.27±0.4	0.55±0.1	1.40±0.02
Ghandy	30.78±0.2	34.24±0.2	25.86±0.3	1.27±0.2	1.24±0.04
Beygom	34.11±0.2	29.30±0.2	21.37±0.3	1.29±0.1	2.19±0.04
Torghabeh Sabz	32.68±0.4	32.25±0.4	22.70±0.8	1.40±0.2	2.02±0.04
Bokhara	34.02±0.3	30.03±0.4	21.25±0.8	1.32±0.1	1.21±0.03

The highest value of flavor index (TSS/TA) was recorded in Torqhabeh Sabz and Ghandy cultivars. No significant difference was observed between other cultivars. The changes in the flavor index of the studied plum fruits ranged from 20.51 (Ghatretala) to 41.21 (Ghandy).

Vitamin C changes in the evaluated cultivars ranged from 2.53 to 9.24 mg per 100 g of fresh weight. This is lower than the amount of ascorbic acid reported by the USDA (2012) for plums at about 9.5 mg per 100 g fresh weight of fruit. Falati et al. (2017) also reported the range of vitamin C changes in plum cultivars and genotypes about 7.04 - 18.5 mg per 100 g of fresh weight. On the other hand, Hajilou et al. (2013) measured lower levels of ascorbic acid for the plum cultivars (4.33 - 4.17 mg per 100 g).

The range of antioxidant activity in the cultivars evaluated in this study was 14.97 to 73.36 percentage. Ghatretala and Rotaby cultivars had the lowest level of antioxidant activity with significant differences from other cultivars. Previously, the percentage of antioxidant activity of plum cultivars has been reported between 96.3 - 11.6 percentage (Falati et al., 2017) and 82.8% (Hajilou et al., 2013).

Total flavonoid changes were between 452-967 mg quercetin per 100 g fresh weight, although there was no significant difference between the cultivars in this experiment. Also, the concentration of total flavonoids in plum cultivars has been reported to be around 118 to 237 (mg/100 g of fresh fruit) (Donovan et al., 1998), which is much lower than the cultivars evaluated in this study.

Table 2- Biochemical properties of plum cultivars

Cultivars	TSS/TA	Ascorbic acid (mg/100 g)	Antioxidant (%)	Flavonoid (mg/100 g)
Ghatretala	20.516±0.4	2.53±0.1	14.97±2.8	452.2±8.7
Pivezhan	27.521±1.9	9.24±0.5	23.51±0.5	674.8±15.2
Rotaby	23.133±0.5	4.511±0.5	30.97±6.7	477.8±7.7
Ghandy	41.217±1.0	5.94±0.4	65.23±1.3	904.8±7.4
Beygom	34.976±0.9	7.15±1.3	73.36±1.2	967±8.9
Torghabeh Sabz	41.157±1.1	8.69±0.1	69.78±1.2	964.8±14.8
Bokhara	36.574±1.1	4.84±0.3	51.55±3.7	881.6±24.0

Conclusion

The high diversity of cultivars studied in this experiment in terms of physical and biochemical properties was one of the most important results that can be used in the introduction, reproduction and breeding projects.

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EFFECT OF BIO, ORGANIC AND INORGANIC FERTILIZATION ON POTATO GROWTH AND PRODUCTIVITY IN PALESTINE

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Abstract

A bio, an organic and an inorganic fertilization trial was carried out on potato (*Solanum tuberosum* L.). Nine treatments were investigated: fertilization program recommended by Ministry of Agriculture (MoAP) 200N + 150P_2O_5 + 300 K_2O Kg Ha^{-1} + 3m^3 poultry manure (PM) as a control (T1), 75% MoAP + biofertilizer (T2), 50% MoAP + biofertilizer (T3), 75% MoAP (25% NPK + 75% PM) + biofertilizer (T4), 50% MoAP (25% NPK + 75% PM) + biofertilizer (T5), 100% PM + biofertilizer (T6), 75% MoAP (25% NPK + 75% compost) + biofertilizer (T7), 50% MoAP (25% NPK + 75% compost) + biofertilizer (T8), 100% compost + biofertilizer (T9). Results revealed that T6, T2, and T4 insignificantly had longer plants while T6 produced the significantly highest number of stems, the weight of the plant, and the insignificantly heaviest tuber. T6 and T7 showed the significant highest leaf nitrogen content and T7 had the significant highest leaf phosphorus content. All treatments insignificantly decreased tuber diameter, yield, number of tuber and leaf potassium content than control, where tuber length, tuber specific gravity, dry matter, tuber starch, total protein, tuber grades, and total leaf chlorophyll were not significantly affected. It could be recommended to apply 100% Poultry manure(5 ton $Donum^{-1}$) + biofertilizer on the potato crops.

Keywords: *Bio-fertilization, manuring, potato yield, tubers, leaves.*

Introduction

Potato (*Solanum tuberosum* L.) belongs to the family *Solanaceae*, is a popular food and is a cheap source of energy, vitamin B, C, and carbohydrates (Powon *et al.*, 2005). Spunta cv. is locally rated as the first cultivar where it is cultivated in two seasons (autumn-winter and winter-spring seasons) with an area of 1.4018 ha in Gaza Strip. This constituted 24.6% of the vegetables open-field cultivated area, produced 3.303 tons $Donum^{-1}$ (MoA, 2013). Although applying mineral fertilizers is essential for plant growth and yield productivity, it led to environmental pollution i.e. leaching a considerable amount of nitrogen in groundwater especially NO_3, and increasing nitrate accumulation in plant organs (EL-Desuki *et al.*, 2010 and Shaheen *et al.*, 2013). So, a substation of mineral fertilizers by manures or combinations of bio, organic, and inorganic fertilization may to lead up preserve soil fertility (Sarhan *et al.*, 2011). No single source is capable to supply the required amount of plant nutrients, where the integrated use of all sources of plant nutrients is a must to supply balanced nutrition to the crops (Arora, 2008). Plant nutrients are essential for the production of crops and healthy food for the world's expanding population. Plant nutrients are therefore a vital component of sustainable agriculture. Increased crop production largely relies on the type of fertilizers used to supplement essential nutrients. The nature and the characteristics of nutrient release of chemical, organic, and biofertilizers are different, and each type of fertilizer has advantages and disadvantages on crop growth and soil fertility (Chen, 2006). Biofertilizers with chemical

fertilizers may be useful to reduce environmental pollution (Ghaderi-Daneshmand *et al.*, 2012). This study aimed to clarify the effect of bio, organic and inorganic fertilization on potato crops under the Gaza Strip condition to replace chemical fertilizers by bio-manuring alternatives.

Material and Methods

This trial was carried out on potato under the open field conditions during two winter-spring seasons in 2012 and 2013 at the Agricultural Research Station of Ministry of Agriculture (MoA) in the Beit Lahya region, North Gaza Governorate, Gaza Strip, Palestine. The soil samples were physically and chemically analysed (Table, 1) before sowing as described by Chapman and Pratt (1961). Also, chemical analysis was carried out on the well water of irrigation (Table 2) and the used manures (Table 3). The weight of Compost (a mixture of municipal organic waste, agricultural waste and animals manure), poultry, and NPK quantity were added depending on the recommended dose of nitrogen (organic and inorganic sources) by the (MoAP). Manures as poultry and compost were applied during soil preparation, where the first quantity of mineral fertilizers as ammonium sulphate (21% N), superphosphate (25.6% P2O5) and potassium sulphate (48% K2O) were applied during soil preparation. The remaining nitrogen and potassium quantities were side-decreased in three equal portions after 30, 45, 60 days of planting (DOP). Biofertilizer (a commercial product called BIOFARM, which is containing photosynthesis bacteria, lactic acid bacteria, yeast and actinomycetes) was applied 4 times after 0, 20, 40, 60 DOP and the other horticultural practices were carried out as recommended by MoA.

Table 1. Chemical properties of sandy clay loam soil

Unit	ds/m		Cations						Anions				
			%	ppm	ppm	me/l	ppm	me/l	ppm	me/l	%	%	
Season	E.C.	Ph	Total N.	P	B	Na+	K+	$Ca^{+2}+ Mg^{+2}$	$NO_3^- - N$	Cl^-	M.O	$CaCO_3$	SAR
2012	2.78	7.6	0.168	229.2	-	2.113	66.6	1.943	7.743	3.672	0.065	-	2.143
2013	0.856	6.5	-	61.5	-	17.478	21.96	-	-	1.7825	-	-	-

Table 2. Chemical analysis of irrigation water

E.C.	pH	Nitrate	Anions mq/L	Cations mq/L		
			CL-	Ca++	Mg++	K+
1.2	6	34.325	77.39	132	143.2	1.51

Tables 3. Chemical analysis of poultry manure and compost

Season		Dry matter%	Organic matter%	Total Solid%	TN%
2012	Compost	69	52.83	47.17	1.1
	Poultry manure	49.81	51.19	48.81	2.1
2013	Compost	66	54.31	44.79	1.3
	Poultry manure	49	52.01	47.19	1.4

Nine treatments were investigated: fertilization program recommended by (MoAP) 200N + $150P_2O_5$ + $300K_2O$ Kg Ha^{-1} + $3m^3$ (PM) as a control (T1), 75% MoAP + biofertilizer (T2), 50% MoAP + biofertilizer (T3), 75% MoAP (25% NPK + 75% PM) + biofertilizer (T4), 50% MoAP (25% NPK + 75% PM) + biofertilizer (T5), 100% PM + biofertilizer (T6), 75% MoAP (25% NPK + 75% compost) + biofertilizer (T7), 50% MoAP (25% NPK + 75% compost) + biofertilizer (T8), 100% compost + biofertilizer (T9). Potato plants were sown in February 28^{th} , 2012 and February 9^{th} , 2013 spaced at 0.25 x 0.7m. The completely randomized block design with four replications was arranged where, the area of each replicate-plot was 9.1 m^2.

Plants were spaced at 70 x 25cm where drip irrigation system was used. Harvesting of tubers was carried out after 100 DOP in both seasons.

Measurements:

Vegetative growth: After 70 DOP, ten plants from each replicate-plot were devoted to determine the plant height, number of main stems plant^{-1}, plant fresh weight. Tubers properties: Twenty plants were used harvested after 90 DOP where a sample of 30 tubers replicate^{-1} were devoted to determine tuber diameter and length, tuber weight, Tuber chemical properties as dry matter% was determined by drying tuber pieces at 70C^{0} for 48 hours (Al Sahaf ,1989), starch content% was calculated (AOAC,1975) using the equation: Starch% =17.55 + 0.89 (dry matter% -24.18). Yield: After 100 DOP yield as tubers weight ton Ha^{-1} were determined where tubers were sorted into different grades (>55and 55-45mm). Leaf chemical content: The 5th leaf from the plant top was used to determine total chlorophyll and NPK after 75 DOP. Total chlorophyll content was determined in fresh leaf (AOAC, 1975). Leaf microelements were measured in samples oven-dried at 70 C0 for 48 hours where nitrogen was measured using micro-kjeldahl (John, 1970), phosphorus was calorimetrically determined (Trough and Mayer ,1939) and potassium was measured by the flame photometer (AOAC, 1975).

Statistical analysis: Data were statistically analysed using Costat program where Duncan's multiply rang test was used to compare between means. Means followed by the same alphabetical letters are not statistically different at $P = 0.05$ (Steel and Torrie, 1980).

Results and Discussion

Vegetative growth (Table, 4) showed that the significant highest plants in T6 in the first season while T8 and T7 respectively came to the significant shortest plants. The number of stems plant^{-1} was significantly higher in T6 compared with the control (T1) and the same with other treatments in the first season where control had the lowest number. Plant fresh weight also was significantly higher in T6 than the other treatments or control in the first season. However, the results show insignificant changes between all treatments in the second season. This might be due to the increases in the soil microbial flora resulted from applying biofertilizer treatments. This microbioflora was able to affect the solubility of low dissolvable inorganic acids. Also, some bacterial species have mineralization and solubilization potential for organic and inorganic phosphorus, respectively (Hilda and Fraga, 2000 and Khiari and Parent, 2005). These results are in agreement with Ahmed *et al.,* (2009), Othman *et al.,* (2011), and Dadashzadeh et *al.,* (2013), In contrast, Farag *et al.,* (2013).

Table 4. Effect of bio, organic and inorganic fertilization on potato vegetative growth.

Treatment	Plant height (cm)		Number of stems plant^{-1}		Plant fresh weight (g)	
	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
T1	39 bcd	38.3 a	2.7 cd	1.6 a	218 cd	266 a
T2	44.9 ab	34.8 abc	3.5 abc	1.6 a	256 bc	229 a
T3	37.3 cd	32.5 abc	2.8 bcd	1.7 a	201 cd	225 a
T4	45.5 ab	33 abc	3.6 ab	1.5 a	300 ab	211 a
T5	39.8 bc	34 abc	3.3 abc	1.7 a	222 c	229 a
T6	48.4 a	35.8 ab	4.1 a	1.5 a	346 a	256 a
T7	38.4 bcd	30 bc	2.8 bcd	1.5 a	189 cd	188 a
T8	32.5 d	29.4 c	2.2 d	1.6 a	145 d	185 a
T9	42.9 abc	33.5 abc	3.2 bc	1.7 a	237 bc	216 a

* Means followed by same letters are not significantly different at p=0.05

Tuber properties (Table, 5) clears that the diameter of tuber was significantly lower in T7 than T9 where insignificant changes were noticed among the other treatments in the first season. The control T1 had the significant highest diameter in the second season except that of T9. Tuber length did not change significantly in both seasons where T6 produced the significant shortest tubers in the second season only. Tuber weight produced the heaviest tuber in T6 for the first season where this increase was higher than T8, T7, and T9 respectively. Dry matter and starch content% were not affected significantly in both seasons. The enhancing application effect of bio, organic and inorganic fertilizers combination on tuber quality diameter could be referred to the role of free-living bacteria on N-fixation in the soil and the role of PDB on increasing the available-P in the soil (Rai, 2006). Combining the mineral fertilizers with the organic manures and biofertilizer could supply the plants with sufficient available nutrients and reduce the leaching of the elements that led to increase the dry matter. On the other hand, this increase in dry matter % by integrated application of bio and organic PM may be attributed to enhanced nodulation, higher N fixation rate, and general improvement of root development (Erum & Bano, 2008). The increase in starch% may be due to an increased supply of nutrients in general and potassium in particular. Potassium plays an important role in the activation of starch synthetase, and also helps in translocation of starch from leaves to tubers (Sud *et al.,* 1992 and Shambhavi & Sharma, 2008). These results are confirmed by AbuEl-Hussein *et al.,* (2002) and Jaipaul *et al.,* (2011).

Table 5. Effect of bio, organic and inorganic fertilization on tuber properties

Treatment	Tuber diameter (mm)		Tuber length (mm)		Tuber weight (g)		Dry matter%		Starch%	
	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
T1	55.9 ab	70.0 a	107.7a	116.0a	144.6 ab	185.3 a	16.96 a	20.39 a	11.12 a	14.18 a
T2	55.1 ab	60.42 b	109.9 a	110.8 a	146.5 ab	174.2 ab	15.57 a	18.83 a	9.87 a	12.78 a
T3	56.10ab	60.86 b	109.7 a	114.7 a	149.8 ab	178.4 ab	14.86 a	19.34 a	9.25 a	13.24 a
T4	54.29ab	60.9 b	119.2 a	115.0 a	150.7 ab	177.3 ab	14.06 a	20.21 a	8.53 a	14.01 a
T5	56.65ab	58.37 b	113.7 a	112.4 a	143.6 ab	172.9 ab	16.43 a	18.28 a	10.64 a	12.29 a
T6	55.74ab	59.73 b	109.8 a	108.3 b	167.1 a	172.4 ab	14.36 a	20.37 a	8.80 a	14.16 a
T7	51.81 b	60.79 b	103.8 a	110.6 a	134.4 b	158.4 b	13.89 a	19.30 a	8.38 a	13.21 a
T8	54.90ab	58.71 b	109 a	110.8 a	128.2 b	163.8 ab	14.64 a	19.98 a	9.05 a	13.81 a
T9	58.76 a	63.65ab	110.2 a	117.8 a	135.2 b	168.4 ab	17.33 a	19.50 a	11.45 a	13.39 a

*Means followed by same letters are not significantly different at p=0.05

Yield components (Table, 6) shows that yield as ton Ha^{-1} in different treatments decreased than control T1 in both seasons. The yield significantly decreased in T7 and T8 than control T1 in the first season and T7, T9 and T8 respectively than control in the second season. Potato yield as tubers of grade >55 mm width showed that except T6 in the first season the different treatments generally decreased yield than control in both seasons where the decrease was only significant in T7 and T8 respectively in the first season. T6 showed the lowest decrease in the second season where the opposite was true for T7. Yield as tubers of grade 45-55 mm width also showed a trend of insignificant decrease in the different treatments than control in both seasons. T8 and T4 respectively in the first season and T6, T4, and T8 respectively in the second season had the lowest decrease. This might be happened due to a balanced C/N ratio, decomposition, mineralization, availability of native and applied macro and micro-nutrients. All of these might have accelerated the synthesis of carbohydrates and its better translocation from the sink to source that might have led to an improvement in yield and yield-related attributes (Malik, et al., 2011). The aforementioned results are in harmony with those found by Abdel-Salam and Shams (2012), and Sood, (2007), but it contradicts with Mirshekari *et al.,* (2013). Nevertheless, Sayed *et al.,* (2015) reported that total yield $plot^{-1}$ treated with compost at 23.8 t ha^{-1} alone or plus biofertilizer insignificantly differed than control.

Table 6. Effect of bio, organic and inorganic fertilization on potato yield components

Treatment	Tubers yield ton Ha^{-1}		Tubers >55mm g $plant^{-1}$		Tubers 55-45mm g $plant^{-1}$	
	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
T1	45.05 a	57.38 a	518.0 a	788.7 a	199.2 a	129.5 a
T2	34.57 abc	49.52 abc	413.9 ab	744.9 a	151.8 a	98.5 a
T3	30.55 abc	47.77 abc	380.9 ab	722.9 a	119.0 a	88.5 a
T4	40.40 abc	51.42 abc	476.8 a	756.7 a	175.5 a	110.9 a
T5	36.36 abc	49.69 abc	436.8 ab	733.7 a	149.7 a	102.1 a
T6	40.97 ab	53.04 ab	543.5 a	774.6 a	136.1 a	122.2 a
T7	25.03 c	42.39 c	255.5 b	611.2 a	144.2 a	104.4 a
T8	26.74 bc	45.08 bc	237.2 b	647.5 a	186.6 a	110.4 a
T9	30.48 abc	44.37 bc	355.9 ab	648.3 a	125.8 a	98.6 a

* Means followed by same letters are not significantly different at p=0.05

Leaf chemical contents (Table, 7) did not show significant changes in leaf chlorophyll content among all treatments or control in both seasons. Leaf N content significantly increased in T7 and T8 in the first season than the other treatments and control where T6 had the highest content in the second one. Leaf P decreased significantly in the first seasons in T2 than other treatments or control. However, no significant differences could be detected in the second season. K content in leaves significantly decreased in all treatments than control T1 where T9 had the significant lowest K in the first season. Also, the different treatments were not significantly different where T7, T9, and T8 respectively had the lowest leaf K content. Using bio, organic, and inorganic fertilizer combination improved leaf total chlorophyll content. This resulted from the increased uptake of NPK and symbiotic activity of nitrogen fixation required for plant growth and development. This is due to the positive correlation between chlorophyll content and N application (Guler, 2009). These results were in agreement with Abdel-Salam and Shams (2012), El-Quesni *et al.* (2013), and Jaipaul *et al.* (2011).

Table 7. Effect of bio, organic and inorganic fertilization on leaf chemical contents of potato

Treatment	Chlorophyll mg $100g^{-1}$ fw		N%		P%		K%	
	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
T1	45.9 a	50.2 a	3.50 cd	4.24 ab	0.82 ab	0.768 a	6.39 a	5.40 ab
T2	50.6 a	57.6 a	3.34 d	4.06 ab	0.379 c	0.668 a	5.45 bc	5.83 ab
T3	45.1 a	45.6 a	3.64 cd	4.03 ab	0.855 ab	0.529 a	5.84 b	5.61 ab
T4	47.3 a	52.4 a	3.81 bc	4.04 ab	0.645 b	0.719 a	5.72 b	5.03 ab
T5	41.9 a	50.7 a	3.75 bc	4.15 ab	0.81 ab	0.753 a	5.12 cd	5.55ab
T6	46.7 a	48.8 a	3.63 cd	4.64 a	0.81 ab	0.639 a	5.51 bc	5.46 ab
T7	48.7 a	52.8 a	4.22 a	3.90 ab	0.883 a	0.750 a	5.71 b	4.90 ab
T8	39.5 a	47.3 a	4.07 ab	3.72 b	0.79 ab	0.563 a	5.24 cd	4.22 b
T9	45.0 a	52.5 a	3.33 d	3.87 ab	0.767 ab	0.552 a	4.90 d	4.88 ab

Means followed by same letters are not significantly different at p=0.05

Conclusion

In conclusion, environment-friendly biofertilizers are good tools to improve the growth and productivity of potato and to reduce the rate of applied chemical fertilizers. It could be recommended to apply 100% poultry manure (50 ton Ha^{-1}) + biofertilizer on the potato crops.

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DETERMINATION OF GENETIC DIVERSITY OF PEAR "PYRUS SYRIACA. BOISS" GENOTYPES AT THE SOUTH OF SYRIA BY USING THEIR MORPHOLOGICAL TRAITS AND MOLECULAR MARKERS

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Abstract

This investigation was conducted in Sweida governorate- Syria to study the genetic diversity and relationships among *Pyrus syriaca* Boiss. genotypes and local pear cultivars depending on morphological traits and SSR molecular markers. The results showed the existence of seven wild pear genotypes belonging to *P. syriaca* Boiss. with different morphological traits of their shoots, leaves, fruits, growth habit and phenological stages distributed at altitude 900-1600 m. The obtained wild pear genotypes were evaluated depending on desirable traits of seedlings that resulted from seed propagation and three suitable genotypes were selected to be used as pear rootstocks. SSR molecular markers were applied on the three selected pear genotypes and three local pear cultivars to determine the genetic relationship among them using 15 selective primer pairs. Fourteen primer pairs were able to amplify 45 alleles, 41 of them were polymorphic alleles with polymorphism percentage 91.1%. The number of alleles per locus ranged between 1-6 alleles with an average 3.2. The highest percentage of genetic similarity was between G3 wild genotype and Meskawi cultivar (0.542), while the lowest percentage was between the local cultivars Romi and G1 genotype (0.219). Cluster analysis divided the studied genetic resources into two clusters; the first cluster contained only Romi cultivar, while the second cluster included all other studied cultivars and genotypes. Ten primer pairs of 14, revealed 24 unique alleles (21 positive and 3 negative alleles). The average of heterozygosity H_e was 0.26. Consequently, SSR technique was powerful tool to identify all investigated pear genotypes and the ability to use it in the breeding and genetic improvement programs.

Key words: *Pear, P. syriaca, genetic diversity, morphological evaluation, SSR molecular technique.*

Introduction

Syria is the origin of many genetic resources of fruit trees where they grow and produce well and tolerate the inappropriate climatic conditions. In this resources fruit trees exist either in the form of independent populations or overlapping with different forest trees, growing on many types of soils and at different altitudes. These trees give a large gene pool of desired characters that increase its resistance to biotic and abiotic stresses. The preservation of genetic resources and genetic diversity is justified, especially after the development of genetic engineering and its applications in the field of genetic improvement. Hence, it is clear the great dimension of the conservation of natural ecosystems and wild species and the essential role they play in sustainable development. *Pyrus syriaca* Boiss is one of the most spread species in Syria, Lebanon, Iraq and Turkey (Mouterde, 1970). Studies indicated that pears are one of the oldest fruit species dating back 3,000 years, and 22 species have been identified (Wu *et al.*, 2013). Ferradini *et al.* (2017) indicated that some species of pears that spread in the Mediterranean basin may return to the Middle East as a basis for their development. Several studies have been carried out to survey wild pear genotypes in Syria and to study the viability of seeds and seed propagation (Muzher, 1998; Ismaiel, 2014). Eskif (2011) stated

that the stratification of Syrian pear seeds has a positive effect on improving germination as the stratification of seeds to 60 days at a temperature of 4°C ±1, gave a 100% germination rate when grown at 20°C. Despite the interest in identifying and characterizing wild Syrian pears genotypes, they have not received sufficient genetic studies (Muzher, 2014). Based on the foregoing, it was necessary to know the reality of the genetic diversity of Syrian wild pears, where the southern region (Sweida) was identified in this study due to the great biodiversity that characterizes it, which requires a deep and comprehensive survey, and the need to benefit from the genetic resources which form an important gene pool and the investment of desired traits to introduce them in the breeding and genetic improvement programs. Therefore, the aim of the research was to survey and characterize the Syrian wild pears genotypes and some local cultivars in the southern region of Syria (Sweida governorate), and to select the appropriate genotypes to use them as rootstocks for the propagation of commercial cultivars.

Material and methods

The research was carried out in Sweida governorate, which is located at an altitude from 900 to 1800 m, and the average annual rainfall is 525 mm. The soil is composed of clay loam, free of calcium carbonate, rich in phosphorus, and low in content of organic material and nitrogen, tends to acidity with a pH of between 6.5 and 6.9.

Plant material

- Wild Syrian pears: The study included seven *P. syriaca* genotypes: G1, G2, G3, G4, G5, G6, and G7). These genotypes are trees or shrubs, up to 7 m in height Their leaves are simple, flowers are white, fruits can be either spherical or pears in shape, different sizes, containing large size seeds with dark brown color.
- Local cultivars: Three local common cultivars were used: Meskawi (Me), Mokh Al-Baghel (MB) and Romi (R).

Morphological characterization

Morphological characterization of the wild Syrian pears genotypes includes 31 traits of shoots, leaves and fruits. Morphological characteristics were studied as follows:

Shoots: 10 shoots of each genotype were studied to determine shoot length, shoot diameter, color, presence of thorns, lenticels

Leaves: 25 leaves of each genotype were studied to estimate blade length, blade width, leaf area, leaf shape, petiole length, leaf margin, leaf apices, leaf base shape, stipules length.

The leaf area was calculated based on equation (Ajayi, 1990): $S= 0.637x(LxW)$; where S leaf area (cm^2), L: leaf length (cm), W: leaf width (cm)

Fruits: 25 fruits of each genotype were studied in terms of stalk length, stalk diameter, stalk cavity, stalk straightness, fruit length, fruit width, fruit shape, width of calyx cavity, fruit weight, fruit color, intensity of lenticels, core length, flesh color, stone cells, seed length, seed width and seeds number per fruit.

Genetic diversity among *P. syriaca* genotypes depending on morphological traits was achieved by using Principal component analysis and Hierarchical canonical analysis.

Molecular characterization

Extraction of total DNA was performed using CTAB protocol according to (Porebski *et al.*, 1997). Fifteen SSR primer-pairs were used (Guilford *et al.*, 1997; Gianfranceschi *et al.*, 1998) as shown in Table 1.The PCR products were detected by electrophoresis on 2% agarose gel. Alleles size was detected by gel documentation (VILBER LOORMOT Germany) using 100bp DNA Ladder (Promega, U.S.A). The amplified fragments were scored either as present (1) or absent (0). The genetic similarity was estimated according to Jaccard coefficient (Jaccard, 1908). Dendrogram was clustered by cluster analysis using UPGMA method. The expected

heterozygosity of the polymorphic loci (He) was calculated depending on allele frequency (Lorenzo *et al*., 2007).
The software's used through this study were Microsoft EXCEL, SPSS 17 and Past.

Table 1: List of SSR primers, the expected allele size, and annealing temperature

SSR Primer	Primer Sequence (5'------ 3')	Allele size range	Annealing temperature
CH02B10	CAA GGA AAT CAT CAATTA AAG CAA GTG GCT TCG GAT AGT TG	114-157	55-45
CH01F02	ACC ACA TTA GAG CAG TTG AGG CTG GTT TGT TTT CCT CCA GC	168-222	55-45
CH01G12	CCC ACC AAT CAA AAA TCA CC TGA AGT ATG GTG GTG CGT TC	107-186	55-45
CH02C06	TGA CGA AAT CCA CTA CTA ATG CA GAT TGC GCG CTT TTT AAC AT	216-254	55-45
CH01H01	GAA AGA CTT GCA GTG GGA GC GGA GTG GGT TTG AGA AGG TT	107-141	55-45
CH02D12	AAC CAG ATT TGC TTG CCA TC GCT GGT GGT AAA CGT GGT G	175-205	55-45
CH01E12	AAA CTG AAG CCA TGA GGG TTC CAA TTC ACA TGA GGC TG	243-248	55-45
CH01F09	ATG TAC ATC AAA GTG TGG ATT GGC GCT TTC CAA CAC ATC	112-139	55-45
CH02B03b	ATA AGG ATA CAA AAA CCC TAC ACA G GAC ATG TTT GGT TGA AAA CTTG	77-109	55-45
CH02B12	GGC AGG CTT TAC GAT TAT GC CCC ACT AAA AGT TCA CAG GC	124-142	55-45
CH01H02	AGA GCT TCG AGC TTC GTT TG ATC TTT TGG TGC TCC CAC AC	226-252	55-45
CH01E01	GGT TGG AGG GAC CAA TCA TT CCC ACT CTC TGT GCC AGA TC	104-138	60-50
23g4	TTT CTC TCT CTT TCC CAA CTC AGC CGC CTT GCA TTA ATT AC	112	55-45
02b1	CCG TGA CAA AGT GCA TGA ATG AGT TTG ATG CCC TTG GA	238	55-45
NB	AGC TCT CGG CTT CAA TGG TTG TTA GCA TGT GAA ATG TCC GTA AAG TA	326	60-50

Results and discussion

Principal component analysis

Morphological characterization of the Syrian pears *Pyrus syriaca* Boiss genotypes was achieved depending on 31 differential traits of shoots, leaves and fruits. The results of Principal component analysis showed that four main components accounted for 85.95% of the variation among the studied genotypes with values 35.16%, 25.21, 15.73 and 9.85 for the main components PC1, PC2, PC3 and PC4, respectively (Figure 1). The important morphological traits associated with the variant values in the first main component PC1 were: Fruit length, fruit weight, blade length, leaf shape, width of calyx cavity, stalk diameter. The second main component PC2 included the intensity of lenticels on the fruits, stalk straightness, shoot length, stalk length, petiole length. The third main component PC3 included fruit shape, the presence of thorns on shoots, shoot color, leaf area, blade width, leaf shape, and leaf apices; It is clear from this component that leaf traits formed an important element in the differentiation among the studied genotypes. The fourth main component PC4 contained seed length, flesh color, seed width, width of calyx cavity, stalk length, leaf base. Genetic diversity studies indicated the importance of using principal component analysis to reduce the number of morphological traits to differentiate between studied species (Al-Halabi and Muzher, 2015; Chalak *et al*., 2016).

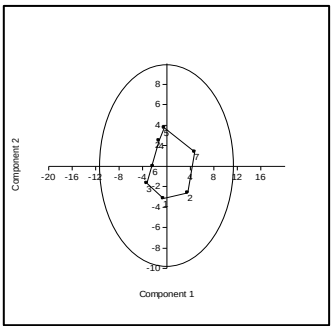


Figure 1: The distribution of Syrian wild pear genotypes based on the first and second main components.

Hierarchical canonical analysis

Hierarchical analysis grouped the studied genotypes into two clusters. The first cluster contains four genotypes which divided into two subclusters, the first one includes G2 and G7 genotypes, while the second one includes G3 and G4 genotypes. The second cluster contains G1, G6, and G5 genotypes (Figure 2). Through these results, it is clear that cluster analysis was able to distinguish between the studied genotypes, and in general, most of morphological traits helped to differentiate among the Syrian wild pears genotypes. Previous studies indicated that cluster analysis based on morphological traits has proven to be an efficient tool in distinguishing between species and cultivars (Aljane and Ferchichi, 2009; Leao *et al.*, 2011).

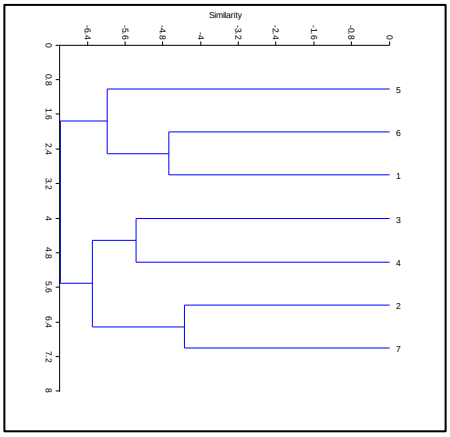


Figure 2: Hierarchical canonical analysis of wild Syrian pear genotypes based on morphological traits.

Selection of wild Syrian pear genotypes with desired traits

Based on the results of morphological characterization and the desired qualities to be provided by the genetic resources nominated for use as rootstocks (no thorns, straightness, lack of suckers formation, seed size, number of seeds per fruit and seeds viability), three wild genotypes have been adopted:

- Genotype G1: Strong-growing trees, thorn-free shoots, large fruits, maturing date on August, large seeds, average number of seeds per fruit 8 seeds, and seeds vitality 100%.
- Genotype G3: Strong-growing trees, shoots with low-content of thorns, medium-sized fruits, maturing date during October, large seeds, average number of seeds per fruit 6 seeds, and seeds vitality 100%.
- Genotype G6: Strong-growing trees, thorn-free shoots, medium-sized fruits, Maturity date on October, large seeds, average number of seeds per fruit 6 seeds, and seeds viability 100%.

Molecular characterization

14 primer pairs were able to detect polymorphism among wild Syrian pear genotypes and local cultivars, giving 45 alleles, 41of them were polymorphic (91.1%), which reflect the ability of SSR technique to distinguish between individuals (Martinez *et al.*, 2003). Primers showed different sizes than expected except for the BN primer, where the allele size ranged from 80bp for CH02B03b to 366bp for BN, due to the fact that these primers are designed from apple genome, and therefore the resulting sizes are different (Yamamoto *et al.*, 2002), many studies have confirmed the possibility of using the primers derived from apple genome to identify other species belong to pomoideae subfamily, including pears (Moghadam and Zarei, 2018). Alleles number ranged from 1 to 6 alleles with an average 3.2 alleles per locus.

Genetic similarity among the studied genotypes and cultivars based on Jaccard coefficient ranged from 0.219 for Romi cultivar and G1 genotype, and 0.542 between Meskawi cultivar and the G5 genotype (Table 2). It is clear that Romi cultivar revealed low genetic similarity with all Syrian pear genotypes (0.292), which requires more genetic studies to verify the origin of Romi cultivar. On the other hand, G1 revealed low genetic similarity with G3 (0.241). However, wild pears genotypes showed a significant genetic variation, which is related to the hybridization that occurs between these genotypes and the role of animals and birds in transporting seeds from one place to another (Wolko *et al.*, 2015).

Table 2: Genetic similarity among wild Syrian pear genotypes and local cultivars according to Jaccard coefficient

	G1	**G3**	**G5**	**Me**	**MB**	**Romi**
G1	1					
G3	0.241	1				
G5	0.50	0.435	1			
Me	0.429	0.308	0.542	1		
MB	0.367	0.346	0.357	0.444	1	
R	0.219	0.269	0.333	0.370	0.267	1

Cluster analysis divided the studied genotypes and cultivars into two clusters (Figure 3), the first cluster includes only Romi cultivar, while the second cluster contains the other genotypes and cultivars which are grouped into three subclusters. The first one included only G3 genotype, the second one included MB cultivar, while the third one contained all the studied genotypes and cultivars. Hence, the importance of cluster analysis arises in the separation of studied genotypes according to their origin and pedigree (Amirbakhtiar*et al.*, 2006).

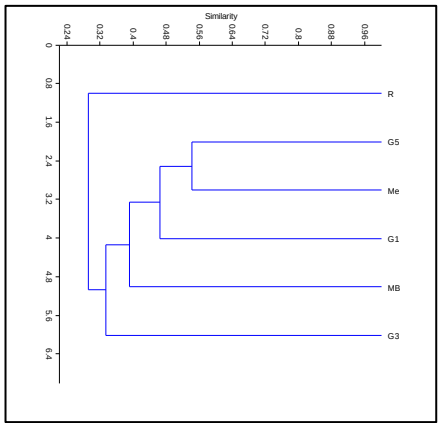


Figure (3) :Cluster analysis as revealed by SSRs data

Unique DNA markers obtained by SSR marker were used in the present study to characterize the six pear genotypes. A total of 10 SSR primer-pairs out of 14 revealed 24 unique SSR alleles (21 positive and 3 negative). Romi cultivar characterized by the highest number of unique SSR alleles which were 10 unique alleles (8 positive and 2 negative). The average of expected heterozygosity (H_e) for SSR marker was 0.263, which reflects the efficiency of this marker for polymorphism detection.

Conclusion

Obtained results showed the highest genetic diversity of *Pyrus syriaca,* which assess a valuable platform of economic importance of genetic resources. Furthermore, the efficiency of SSR technique to detect the genetic variation among the studied genotypes, and its importance in breeding programs.

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SELECTION OF NEW APPLE ROOTSTOCKS DEPENDING ON LOCAL GENETIC RESOURCES IN SYRIA

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Abstract

This investigation is part of apple rootstock breeding program conducted at General Commission for Scientific Agriculture Research – Pome and Grapevine Division in Sweida governorate (Syria). The objectives were to select new apple rootstocks appropriate for local environment (tolerance/resistance to pests, diseases and abiotic stress factors), and to detect some desirable attributes using molecular markers. The work was performed by using 9 local genetic resources (5 seedling genotypes, 3 local apple cultivars and hybrid of the local apple cultivar 'Skarji' and MM106 rootstock). The obtained results led to select 3 genotypes (Sukari Alswieda 'S', Alswieda1 'B' and Skarji Alswieda 'H') as rootstocks which revealed the standard attributes of rootstock, and showed a high genetic similarity among the seedlings belong to the same mother plant, and high percentage of successful budding with commercial cultivars observed as well. 'S' rootstock was classified as vigorous rootstock, it could be propagated either by seeds or vegetative propagation, efficient root system is producible in either way. Moreover, 'S' rootstock produced the highest number of seedlings which revealed 4 drought tolerant genes. 'B' rootstock was classified as a moderate vigor rootstock, and it could be propagated by seeds. 'H' rootstock was classified as a moderate to vigor vegetative rootstock, showing phenotypic and genetic resistance to woolly apple aphid. Consequently, the use of the local selected rootstocks is necessary to improve apple cultivation in Syria.

Key words: *apple, Malus sp., seedling and vegetative propagated rootstocks, rootstocks tolerant to drought.*

Introduction

Apple grows as a combined tree which consists of rootstock and scion. The rootstock is either seedling or vegetative (Wertheim and Webster, 2003). The apple rootstocks are used as an easy way of propagating scion cultivars. Apple growers depend on seedling rootstocks, only in a few countries where it is either hard to use vegetative rootstocks, or not economical (Webster and Wertheim, 2003). Seedling rootstocks can have some advantages over vegetative rootstocks, as seedling rootstocks are easy and cheap in propagation, virus free, and free of soil rots that infect roots. Moreover, it is possible to eliminate wide genetic diversity through using defined sources for seeds such as the cultivar Red Delicious in America, cultivars Antonovka and Bittenfilder in Europe, and the wild species in China and Japan (Wertheim and Webster, 2003).

Rootstocks play an important role in tree growth and tree physiology, therefore rootstocks need to add many other valuable characteristics to the orchard tree, such as growth control, induction of precocious, consistent and abundant yields assistance, and pest, diseases and abiotic stress factors tolerance/resistance (Webster, 2003; Dolgov and Hanke, 2006).

Most apple producing countries such as Germany, Poland and Russia tend to select their rootstocks depending on their local genetic resources adapted to their environmental conditions (Feuerhahn and Jesch, 2000; Czynczyk and Jakubowski, 2007). As a result of climatic changes affecting the growth of fruit trees in the temperate zone, the rootstock

breeding programs focus on producing rootstocks tolerant to abiotic stress as drought, and resistant to biotic stress as pests, to keep pace with sustainable agriculture aimed to minimize the use of pesticides (Hrotko,2007).

In Syria, apple tree is one of the most important trees; it occupies the first level in production of deciduous fruit trees. The most of these trees are grown on mixture of seedling rootstocks. On the other hand, there is a group of vigorous and old local apple cultivars in Syria which are very adapted to local environmental and climatic conditions. These cultivars were used in apple rootstock breeding program in order to achieve the objectives of getting some mothers as seeds sources, and selection of vegetative apple rootstocks which are tolerant/resistant to pests and drought.

Materials and methods

This investigation was achieved during 2008-2016 at Pome and Grapevine Division- GCSAR in Sweida governorate which located in the south of Syria at 1,525 m altitude.

Plant material

- A, B, C, D and E seedling genotypes produced by open pollination, and distinguish by abundant and regular yield.
- Local apple cultivars: S1 (Sukari 1), S2 (Sukari 2) and SK (Skarji): These cultivars are vigorous and their yield is good and regular (Al Halabi, 2007).
- MM106 rootstock: semi vigor rootstock, it has the gene resistance (*Er*1) to wooly apple aphid (Preston, 1955; Preston,1966; Parry, 1965).
- The two commercial cultivars Golden Delicious (used in budding) and Royal Gala (used as a control in tolerant drought genes detection).

Methods

Hybridization

MM106 rootstock flowers were pollinated with the pollen of local apple SK (Skarji). However it is recommended to use standard rootstocks as mothers in breeding rootstocks program, as it has the characters of rootstock (Johnson *et al.*, 2001).

Seed collection

The seeds from fruits of studied genotypes, local apple cultivars and hybrid (H) were collected at physiological ripening stage. Seed vitality (100 seeds in 4 replications), planting seed and field germination percentage were achieved.

Selection stages

1- Selection of genotypes carrying desired attributes depending on: parent's characters (number of seeds per fruit, seed dimension and number of seeds per gram), and progeny characters of each genotype (percentage of plants with erect and clean stem (without thorns), and plants with bend stem and stem with thorns according to Cummins and Aldwinckle (1995).

2- Growth characters of selected genotypes (100 plants of each of them): plant length, plant diameter at 15 cm height, root system characters were determined (main root length, number and length of secondary roots).

3- Budding: 40 seedlings of each genotype were budded with Golden Delicious cultivar, then the percentage of successful buds, the length of resulted plants and roots (main root length, number and length of secondary roots) at the end of growth season were measured.

4- Genetic similarity between the progeny of each genotype with the mother plant using SSR markers.

5- Detection of tolerant drought genes using 5 specific markers (Peptidyl, 18.1kDaHSP, ERC, Xero and COR4) according to Wisniewski *et al.*(2008).

6- Detection of genes responsible for resistance to woolly apple aphid: the field infestation was applied, then, according to Bus et al. (2008), 8 markers-linked to the resistant genes for woolly apple aphid on plants were used, in order to show field resistance to the pest.
7- Stool bed propagation was used to test the ability of selected genotypes (which showed genetic resistance to pests and drought) to vegetative propagation.

Results

Evaluation stages led to excluding 4 genotypes which did not express the desired attributes as a rootstock, keeping 2 genotypes to continue in hybridization process due to their preferable traits, and selecting 3 genotypes as rootstocks as follows:
1- Alswieda1 'B' is an intermediate seedling rootstock which was produced from open pollination. Its seeds are highly vital, with high germination percent. It produces high percentage of plants with erect and clean stems (without thorns). Genetic similarity between progeny and mother plant is 59%,and 76.4% of infested plants with wooly apple aphid were in scale 3, which is classified as medium susceptibility to this pest according to Bus *et al*. (2008). Additional traits of this rootstock are shown on Table (1).
2- Skarji Alswieda 'H' is an intermediate to vigor vegetative rootstock. It was produced from hybridization of MM106 with pollen of local apple cultivar Skarji. This rootstock is distinguished by its resistant to wooly apple aphid. The molecular marker using the linked marker NZsn_O05 proved that this rootstock carries the *Er1*resistance gene to wooly apple aphid. On the other hand, this rootstock produced erect plants with clean stems (without thorns), and good root system in stool bed propagation. Additional traits of this rootstock are shown on Table 1.
3- Sukari Alswieda 'S' is a vigor vegetative and seedling rootstock. It was produced by open pollination of local apple cultivar Sukari. The seeds of this rootstock are highly vital, with high germination percent; seedlings are erect and stems are clean . Genetic similarity between progeny and mother plant is 66%, and 75.5% of infested plants with wooly apple aphid were in scale 3, which is classified as medium susceptibility to this pest according to Bus *et al*. (2008).Moreover, it is differentiated by giving high number of seedlings (75%) that carried the four studied tolerant genes to drought, 16% and 9% of seedlings carried 3 and 2 of studied tolerant genes to drought respectively. Vegetative produced plants in stool bed propagation are distinguished with long and dense roots. Additional traits of this rootstock are shown on Table (1).

Table 1.Traits of selected rootstocks.

Trait	**Alswiea1 'B'**	**Skarji Alswieda 'H**	**Sukari Alswieda 'S'**
Propagation method	seeds	Vegetative (stool bed)	Seeds+Vegetative (stool bed)
Number of seeds per fruit	7	-	6
Seed dimension (mm)	6.8X3.9	-	6.6X3.4
Weight of 100 seeds (g)	3.5	-	2.2
Length of seedling (cm)	61	60	88 – 75
Diameter of seedling at budding area (mm)	7.6	7.5	8.1 - 7.8
Length of main roots of seedling (cm)	32.3	30	39.3– 40
Number of plants per mother in stool bed		6	8

The plants produced from the selected rootstocks were budded with Golden Delicious cultivar. The results showed high percentage of successful buds (90 to 98 %), in addition to the harmony in length and root system between the budded plants of each rootstock.

Discussion

Apple rootstocks need to have general characters, such as ease of vegetative propagation or seed production, erect and clean stems that can be easily budded (Janick *et al.*, 1996). Likewise, these characters are available in our selected rootstocks, as well as production the appropriate thickness for the budding process, as pointed out by Wertheim and Webster (2003) that the rootstocks suitable for budding should be neither thin nor thick15 cm above ground.Furthermore, there is another set of traits should be available in rootstocks some traits help breeder, and others help grower, since the compatibility with commercial cultivars is essential for breeding program (Janick *et al.*, 1996). The selected rootstocks in this research have been tested with Golden Delicious which is a standard cultivar in terms of growth habit and vigor (Toth *et al.*, 1998).Root system is an important index in selecting apple rootstocks, due to the ability of fixing the rootstock, and water and minerals absorption. Atkinson (1983) mentioned that root system of apple trees is consisted of a mixture of different morphological, structural, and age types which are responsible for water and minerals absorption.
The genetic similarity between plants and their parents (Alswieda1 'B' and Sukari Alswieda 'S') have high values. This result indicated that it is necessary to use seeds from defined sources when producing seedling rootstocks to avoid a huge genetic variation. In addition to this, determination of genetic similarity between species and genotypes are crucial in breeding programs, to select parents of high gene pool in hybridization. The importance of determination genetic relatedness in breeding rootstock programs is to regulate germplasms, which in turn directs the selection process through choosing parents to be hybrid (Jin*etal.*,2012). Plant tolerance to drought is related to many biotic, physiological and genetic mechanisms, therefore a better understanding of the tolerance mechanism is to determine the genes responsible for deficit water response, and how these genes express (Amudha and Balasubramani, 2011). Moreover, the perceptive of drought effects on plants is vital for improving management practices and breeding efforts in an agriculture undergoing rapid climate change (Chaves *et al.*, 2003). The use of modern molecular biology tools for improving and elucidating the control mechanisms of abiotic stress tolerance is very essential (Amudha and Balasubramani, 2011). According to that the molecular markers were used in this research to detect some of the genes responsible for drought tolerance in the selected rootstocks. The 4 studied genes play an important role in protecting and conserving the cells during drought stress, and these genes fell mainly into the functional categories of stress (heat shock protein, dehydrins) and photosynthesis (Wisniewski *et al.*, 2008).
MM106 semi vigor rootstock was used in breeding apple rootstocks in Syria, which is resistance to wooly apple aphid. It was used as mother in breeding rootstocks program, according to Johnson *et al.*(2001). The resistant character to wooly apple aphid was successfully transferred to the selected rootstock Skarji Alswieda 'H'. The wooly apple aphid insect (*Eriosoma lanigerum* Hausm.) is considered as an economical pest infests apple. It infests both trunk and root systems. As a consequence trees can die in some severe cases of infestation. Accordingly, the breeding rootstock programs focus on selecting rootstocks resistant to that pest, not only by identifying alternative sources of resistance but through a pyramid structure to develop the trait of resistance as well (Sandanayaka *et al.*, 2003).

Conclusion

The results showed that the rootstocks Alswieda1 'B' and Sukari Alswieda 'S' have the standard traits of seedling rootstock. Rootstock Skarji Alswieda 'H' is resistant to wooly apple aphid. Also, there is probability to propagate rootstocks Sukari Alswieda 'S' and Skarji Alswieda 'H' in stool bed. Moreover, the results revealed the importance of using molecular biology in breeding program particularly in fruit trees, as the application of molecular techniques could reduce the period necessary for obtaining new rootstocks compared to traditional breeding programs.

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A METHOD OF APPROXIMATING THE AREA SWEPT BY A BODY MOVING ON A PLANE

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Abstract

The paper presents a practical method of approximation of the area of the planar zone swept by a body in rotational and translational motion on that plane. Although theoretical solutions might be found, there are reasons why these are not practical for more complicated movements than the pure translational or rotational. First is that of measuring only once the areas of the plane portions several times swept. The second consists of the difficulties of delimiting the areas several times swept by the moving body. The proposed method consists of processing the digitized images from the graphical results of the numerical simulations or the photographs of the "traces" left on the plane of movement by the body in motion. The mathematical model of the planar motion of a rigid solid simulates the movement of working bodies of some agricultural machines that soil tillage, having relative movements to the load-bearing structure. The results presented include the validation of the method for pure translational and pure rotation movements. For the case of the general flat rotational and translational movement, results are given for several sizes of the digitization matrix. Also in the article are highlighted the parameters of the physical process that influence the ratio between the area swept and the area of the translation travel corridor.

Keywords: *swept, area, estimation, image, processing.*

Introduction

The origin of the problem for which a solution is proposed in this article is the need to estimate the area of agricultural land processed by the rotary working bodies of some agricultural machines intended for soil works. Such agricultural machines are various types of cultivators, planters, ploughs between vines, rotary tillers, rotary cultivators, intelligent hoeing machines, etc(www.clemens-online.com; www.alamy.com; www.maschio.co.uk ; www.breviglieri.com), see Fig.1 and 2, for example.



Fig. 1 **Rototiller GRC - rotors and workpieces.**



Fig. 2 **Rototiller GRC - front view, overall.**

Approaches to this problem exist in the literature: (Ling Z. K.1996, Foote R. L 2006). The calculation of the area swept by components of some mechanisms is also encountered in the problem of windscreen wipers, as well as in the theory of wind turbines.

Material and Methods

The material of this study consists of continuous pencils of curves (a family of curves) located on a plane. These pencils are generated by the translational and rotational movement of a body on the plane. In other words, the material of our study consists of the "traces" left on a plane by the motion of a body.

For simple movements, which generate beams that are easily described analytically and without multiple points, calculating the area of the curve bundles can be relatively simple, as will be seen in the results and discussions chapter. When the curve bundles have multiple areas covered, however, the theoretical calculation becomes difficult, because the areas of these multiple points zones must be delimited and estimated. The areas of the multiple planes swept by the moving body are areas that contain points with the higher order of multiplicity or, in other words, the curves that generate such areas are curves with different orders of multiplicity, (Bobancu V.1974, Marcus S., Maza M.,M., Vrbik P.2012).

For these cases, difficult to analyse theoretically, the method of analysing the image of the "footprints" of the body on the plane of motion is proposed. For the case of numerical simulation, graphic images generated by simulation programs of the body movement in the plane will be analysed, and for the case of analysing some real "traces", in the field, will be analysed (in a next stage), images obtained by photographing the modified soil at the surface, by the body movement. The rotational and translational motion is given in all books of theoretical mechanics, for example, (Dragos L.(1983).

Results and Discussion

For clarity, in Fig. 3 is given the geometry considered for the body moving in the plane, generating the area whose surface we seek to estimate.

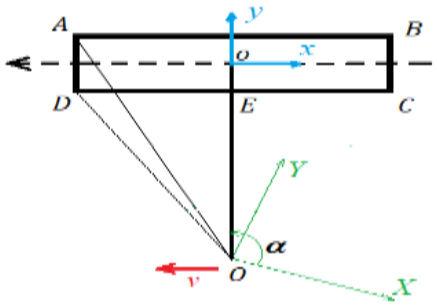


Fig. 3 **Geometry and kinematics of the roto translatory movement of a rectangular body in the plan. The dimensions of the segments are (cross-section of a knife):** $\overline{AB}=\overline{CD}=L, \overline{AD}=\overline{BC}=l, \overline{Oo}=R$.

In the case of a pure rotation, the minimum and maximum radius of the circling beam have the expressions (see Fig. 3):

$$R_{min}=R-\frac{l}{2}, R_{max}=\sqrt{\left(R+\frac{l}{2}\right)^2+\frac{L^2}{4}} \quad (1)$$

First, we give the results of the test of translating a rectangle into a rectangular band, the centre of the rectangle describing the median line of the band. It is considered a rectangle of the length of L cm and width l cm. The translation of the rectangle is made according to the equations:

$$x(X,Y,Z,t) = X + v_{0x}t, y(X,Y,Z,t) = Y, z(X,Y,Z,t) = Z \quad (2)$$

wherein $\boldsymbol{x, y, z}$ are the current coordinates of a point of the rectangle, and $\boldsymbol{X, Y, Z}$ are the initial coordinates of the same point. In the conditions of our theoretic experiment (see Fig. 3), the next conditions are satisfied:

$$-\frac{L}{2} \leq X \leq \frac{L}{2}, R - \frac{l}{2} \leq Y \leq R + \frac{l}{2}, Z = 0. \quad (3)$$

The relations (3) represent conditions for an initial point of coordinates $\boldsymbol{X, Y, Z}$ to belong to the rectangle. For simulation, the dimensions of the rectangle are taken: $\boldsymbol{L}$ = 0.05 m, $\boldsymbol{l}$ = 0.01 m, for the radius $\boldsymbol{R}$ = 0.1 m, and the allocated bandwidth is 0.03 m wide. The rectangle is in translational movement parallel to the sides of the band, with the median line superimposed over the median line of the band. Under these conditions, this rectangle occupies the 1 cm strip inside a 3 cm wide strip, which means that the rectangle sweeps one-third of the surface of the tape allocated for the motion. This means that the ratio between the area swept by the rectangle and the area of the area allocated for movement expressed as a percentage is 33.333%. This is the theoretically expected result for the translation of the rectangle. To draw the points of the rectangle in translation movement, the trajectories of the peaks of the rectangle are drawn with a very small time step, which apparently, leads to the coverage with the desired colour, of the swept area by the rectangle. In this way, is obtaining an image like the one in Fig. 4.

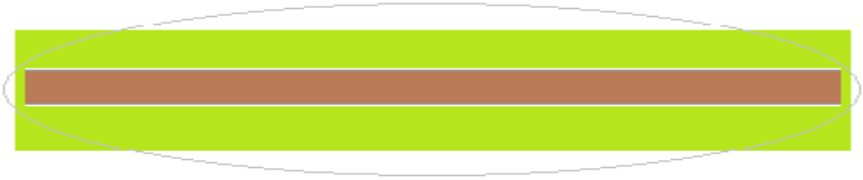


Fig. 4 **The area swept by the rectangle in motion parallel to the boundary of the motion band (pure translational ,brown color) - image obtained by numerical simulation**

The accuracy of the numerical result, obtained by processing the image, depends on the size of the image, on the number of rows and columns of the matrix into which the image is transformed. In this case, the ratio sought (the area swept / the total area of the distributed area), does not depend on the deformations of the image in the two directions, deformations that do not change the angles (homothetic). For example, the generated image (Fig. 4) with the help of the Mathcad program was digitized using the Paint program, depending on the physical size of the image, obtaining different sizes of the matrix resulting by digitization. Some examples are given in table 1, the scanned files being of type BMP or JPG.

Table 1.Estimates of the ratio of the area on images of different physical sizes and with different digitizing matrices for the translation motion

Rows	Columns	The ratio of swept area, %	Error, %	File type
728	1742	33.379	0.139	JPG
729	1741	33.357	0.071	JPG
110	756	35.454	6.365	BMP
113	757	34.513	3.541	JPG
112	758	34.821	4.465	BMP

The differences between the images with the digitization of close dimensions, come from the cut and extract from the initial image, of the area in which the rectangle moves. Similarly, for the rotation of the same rectangle around a fixed point located on the median of the large sides of the rectangle, outside it at a distance $\boldsymbol{R}$ = 0.1 m from the centre of the rectangle, movement is given by the equations:

$$x(X,Y,Z,t) = X + R\cos\omega t\,, y(X,Y,Z,t) = Y + R\sin\omega t\,, z(X,Y,Z,t) = Z \quad (4)$$

where $\omega = n\pi/30$ are the rotation speed and n is the RPM. Using these data, the results from table 2 are obtained.

Table 2.Estimates of the ratio of the areas on images of different physical sizes and with different digitizing matrices for the rotation motion

Rows	Columns	The ratio of swept area, %	Error, %	File type
384	397	18.204	2.991	JPG
728	798	17.608	0.381	JPG
1535	1723	17.354	1.819	JPG
1615	2723	16.444	6.965	JPG
1387	1579	17.724	0.277	JPG

The graphical representation of the trajectories beam described by the points of the rectangle in the rotation is given in Fig. 5. For a better understanding of how to generate the area swept by the rectangle in rotational motion, in Fig. 6 is shown a succession of positions with a greater step, to be able to notice the successive positions occupied by the sides of the rectangle.

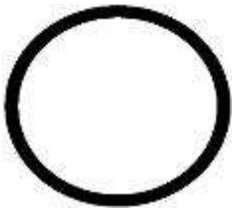


Fig. 5 **Trajectories beam described by the points of the rectangle in rotation**

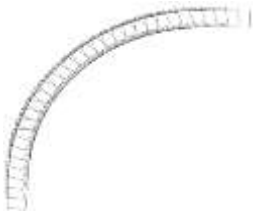


Fig. 6 **Succession of positions with a greater step in time, to be able to notice the successive positions occupied by the sides of the rectangle**

The two examples given above are examples for which we have simple theoretical solutions and we easily compared the numerical results of the proposed method with the theoretical results for validation and estimation of accuracy. The following is an example of a movement commonly encountered to the machine organs of some cultivators. There are working organs of some machines intended for soil works, which have a local rotation motion and combined with the forward movement of the agricultural aggregate, leading to a beam of cycloid-type trajectories. For the simulation, the following equations will be considered, for the rotation motion in the plane:

$$x(X,Y,Z,t) = v_{0x}t + X\cos\omega t - Y\sin\omega t\,, y(X,Y,Z,t) = X\sin\omega t + Y\cos\omega t\,, z(X,Y,Z,t) = 0. \quad (5)$$

A graphical image of the beam of trajectories generated by the rectangle in motion, (5), is shown in Fig. 7, a and b. In reality, the surface worked at each depth is much larger because the breaking of the soil propagates at a large distance from the knives (fig. 1).



a b

Fig. 7 **The beam of trajectories generated by the plane rectangle in the motion of roto translation in the plane, a-theoretically, b-physically.**

A numerical study carried out over time intervals increased successively with each period of the rotation movement, shows that the ratio between the area swept by the rectangle and the area of the aisle is not constant. This phenomenon can be seen on the graph in fig. 8. To apply the above results in practice, the planar rectangle is physically assimilated with a section of a working body of an agricultural machine for working the soil, which is in a flat rotational and translational movement. Two or more such working organs are mounted on the rotors of cultivators. In this way, the degree of soil processing (the ratio between the area of the soil being swept and the area of the corridor determined by the working width of the machine) can reach very high values. The main problem then becomes the minimization of the areas of soil which are multiple processed (over which the working organ or organs pass several times). The precision obtained for estimating the area swept by the plan object in motion is sufficient, considering that, in general, the real trace of the organs in the soil appreciably exceeds the section of the working body.

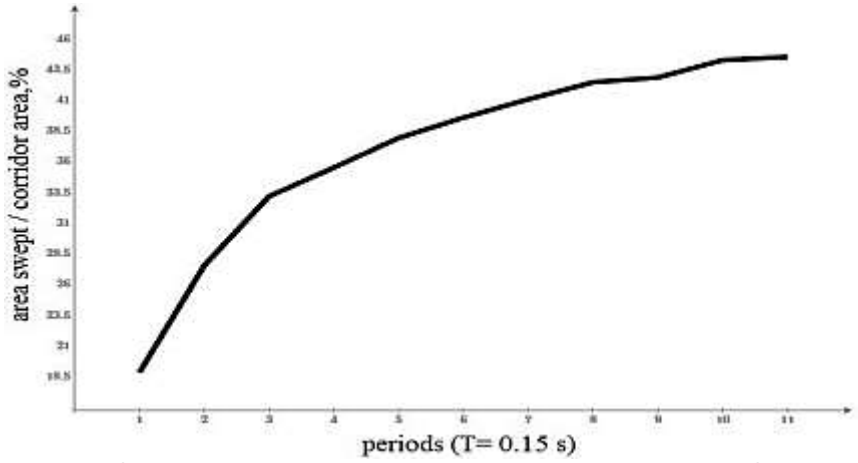


Fig. 8 **Variation of the ratio between the swept area and the area of the rectangular displacement corridor. The period has a value of 0.15 s.**

The simulations performed using the proposed method, show that the main parameters on which the efficiency ratio of the soil processing depends, are: working speed, the rotational speed of the working organs and the geometry of the working organs and their location on the working width of the machine, also the number of knives. Theoretically, one can calculate a measure of the area swept by the body in the rotational movement, considering the minimum and maximum radii of the beam curves and the length of the curve on which the centre of the segment determined by the minimum and maximum radius moves. For the translation movement, this solution will be exact and leads to the 33,333 reports (see table 1). For the pure rotation movement, already at a complete rotation overlap occur.

Conclusions

The results of the numerical experiments (simulations) described above allow to draw some conclusions about the possibility of using the proposed method for practical purposes.
The method of estimating the area of the area swept by a planar object in motion on a plane, although it is not yet very accurate, is useful because it eliminates the areas matured several times by the moving object. Theoretical calculation of multiple areas swept by the moving object is difficult, sometimes even impossible.
The accuracy of the method depends appreciably on the accuracy of the image processing. It is recommended to estimate using jpg or BMP images as large as possible, which will lead to the analysis of the image on arrays of dimensions of hundreds or thousands of pixels. Cropping the image of the displacement corridor from the graphical representation obtained by simulation must be done with care because the elimination of a row or a column automatically leads to estimation errors.
It is found that the main parameters that influence the relationship between the swept area and the area of the moving aisle are: the speed of movement, the speed of rotation of the working body (in the simulation, the rectangle) and the geometrical parameters of the section of the moving object and the cultivator's rotors.
The proposed method for estimating the area of the planar area swept by the object in motion on a plane is prepared, through theoretical experiments (numerical simulations), to be applied in the field. This means trying to estimate the same area of the area swept by the working organs in motion, using photographs of the worked areas.

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EXPERIMENTAL RESEARCH FOR THE DETERMINATION OF FRACTURE EFFICIENCY OF TRACTORS

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Abstract

This paper seeks to determine the braking parameters of agricultural tractors produced in Romania for admission in road traffic on public roads as well as agricultural aggregate tractors, imposed by national, European and international norms, so as to ensure safety and security in the transport, which is verified by the test of: tractor, agricultural trailer, tractor components and trailers (assemblies) that directly compete for safety and security in transport: traction device for wheeled tractors, mating eyes for biax trailer or uniaxial.
In order to verify compliance with the safety and security requirements in transport, experimental research was carried out on a U-774 DT agricultural tractor, a REMO RAB type 4 trailer, a tractor traction device, a towing eye from the trailer . In the tests, several parameters were determined: the determination of the longitudinal braking stability by the calculation of the experimental method, the determination of the braking efficiency, the braking with the parking brake, the braking with the service brake. All the tests performed on the tractors, the most important type approvals are braking efficiency, as it has the greatest impact on road safety.(Beghes H.,Popescu ,S, Popa L 2002; Iordache S,Badescu M ,Boruz S,Vladut V,Eng Popa L 2011).
The tests were carried out on a concrete track designed for braking tests, brake test items were mounted on the tractor, or several attempts were made for service braking and stationary braking at different speeds. The results were interpreted graphically, and centralized tables, conclusions were drawn and made proposals to improve the braking system.

Keywords: *tractor;braking;agricultural trailer;trials*

Introduction

In this paper we aimed to determine the parameters of braking efficiency of tractors manufactured in Romania in accordance with the national and European regulations and regulations in force for admission in road traffic on public roads. Several determinations were made according to RNTR 2/2011, the tests - They performed on a concrete track.(Vladut V,Iordache S,Badescu M,Boruz S,Biris S,Paraschiv G,Dumitru I.,Matache M.,Popa L 2010) The role of the braking system by means of the mechanisms used is to reduce the overall or partial speed of the braking system.(Paunescu D.,Beghes H.,Vladut V,Biris S. 2001).The braking system must meet the following conditions: -to ensure a uniform distribution of the braking energy on the two driving wheels. -Framing must be progressive and safe The tests were carried out on a U-774DT tractor, where the braking system acts on the rear wheels via the transmission rather than directly on the wheel. The braking system is a mechanical drive with direct drive.(Candea I.,s,a 2001,2003,2008).Several attempts were made and the results obtained were tabulated and the corresponding oscillograms were drawn.

Material and Methods

The tests were carried out on a Universal U-774 DT tractor by mounting braking test elements such as:



Fig.1. **U-774 DT tractor**

-in the front you will find a correvit s-400 sensor
-in the front on the top right of the bonnet is positioned through an elastic fixation system the light barrier detector
-Inside the passenger compartment was placed the DAS 3 Data Acquisition System

As a result of the tests, prior to the calibration of the equipment, braking with the service brake and the parking brake will be carried out and the total braking time, average deceleration and maximum deceleration, initial speed, braking distances, pedal operation.(Andreescu C.2010).
Or performed 2 brake servo samples with the parking brake and the results obtained were in the table, followed by the representative oscillograms with the braking parameters of the tractor at the two speeds.(Nedelcu A. 2007).

Results and Discussion

The aim was to determine the braking efficiency of the tractor according to the driving speed, at the same time highlighting the braking space and the maximum deceleration. The force on the brake pedal was measured and after the tests the corresponding diagrams were drawn.

Table 1

PROBE 1 A. Brake with service brake
Brake type: multidisc, in oil bath

Parameters determined		**Maximum allowable values of Directive 76/432 / EEC**	**Average determined values**
Initial speed,	km/h	40 + 3	19,93
	m/s	11.1	5.53
Average deceleration [m / s^2]		-	1.73

Maximum deceleration [m / s2]			1.88
Braking distance (m)	calculated according to D 97/51 / EC *)	$x \leq 0{,}15\dot{x} + \dot{x}^2/116$	6.41
	determined experimentally	-	4.19
Total braking time [s]		-	1.19
Air pressure at coupling braking **)		-	-
Maximum brake cylinder pressure [kPa]		-	-
Pedal power [daN]		60	49.5 when the deceleration first passes through the mean value of the maximum deceleration

*) With cold brakes, (type 0) according to Directive 96/63 / EU to amend Directive 76/432

Representative oscillogram with variation of the U 774 tractor braking parameters at the speed of 19.93 km / h

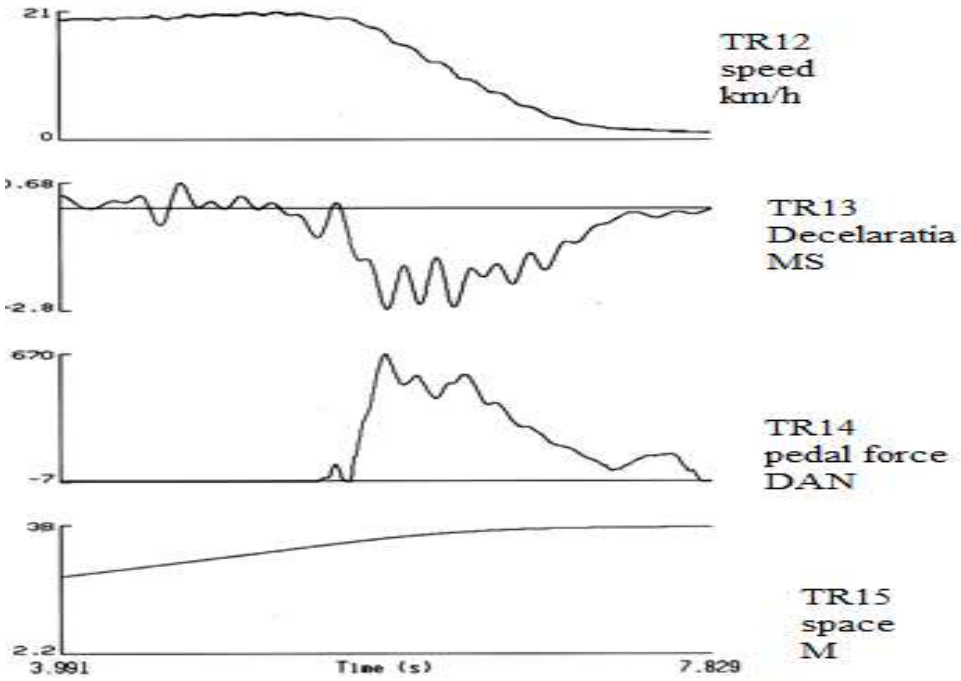
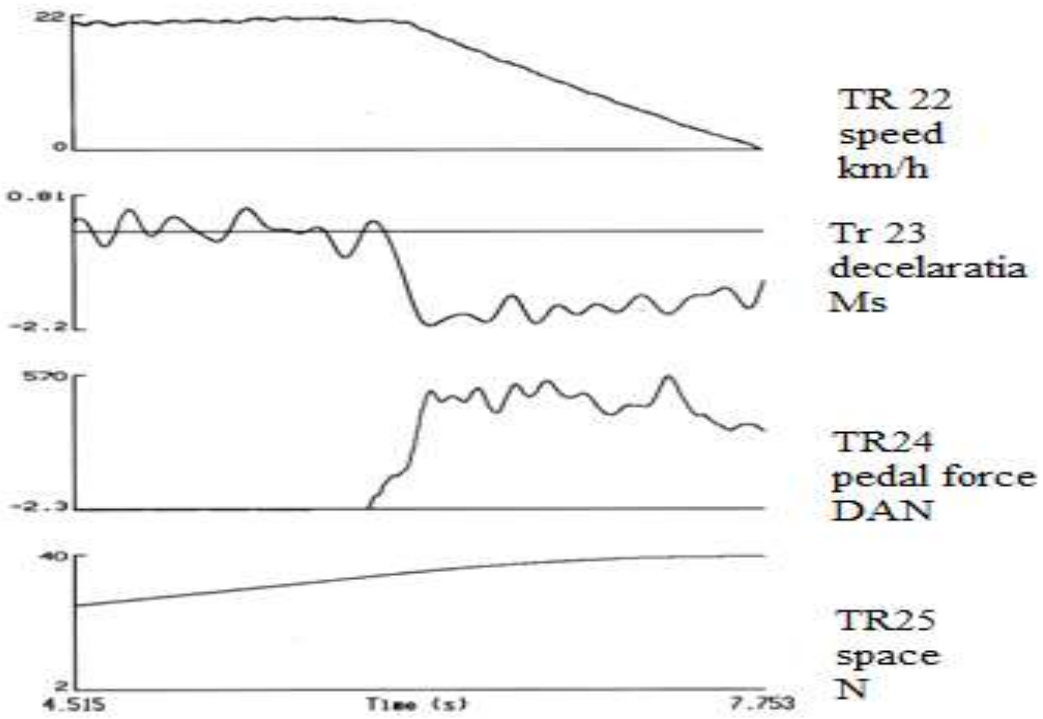


Table 2

PROBE2 A. Brake with service brake
Braking: multidisc, in oil bath

Parameters determined		**Maximum allowable values of Directive 76/432 / EEC**	**Average determined values**
Initial speed,	km/h	40 + 3	20.88
	m/s	11.1	5.8
Average deceleration [m / s2]			1.6
Maximum deceleration [m / s2]			1.72
Braking distance (m)	calculated according to D 97/51 / EC *)	$x \leq 0.15\dot{x} + \dot{x}^2/116$	6.89
	determined experimentally	-	5.43
Total braking time [s]			1.79
Air pressure at coupling braking **)			-
Maximum brake cylinder pressure [kPa]			-
Pedal power [daN]			38.5 where the deceleration first passes through the mean value of the maximum deceleration

*) With cold brakes (type 0) according to Directive 96/63 / EU amending Directive 76/432 / EEC (x 'being the speed in km / h)
**) In the case of tractors equipped with a pneumatic system for braking trailers Representative oscillogram with variation of the U 774 DTtractor braking parameters at the speed of 20.88 km / h



Conclusions

The U 774 DT tractor brakes suitably comply with D 74/432 / EC as amended by D 97/54 / EC.

The average speed and maximum deceleration decreases at higher speeds

At higher speeds the braking distance increases

Increasing the braking time with the increase of the speed of deployment

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EFFECT OF NITROGEN AND PLANT DENSITY ON SOME PROPERTIES OF GROWTH AND YIELD COMPONENTS FOR TWO VARITIES OF SORGHUM BICOLOR L. MOENCH

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Abstract

Effects of different nitrogen levels and plant inter-row spacing on growth, physiological, yield traits and hydrocyanic acid content of two sorghum varieties were studied. The experiment was carried out on the farm of the glasshouses project of Misurata city-Libya. The experimental site was 32016'10.10" N 150 02'49.66" E. Seeds of Sorghum bicolor (L) cv. Azra3 and Azra7. using (RCBD) Split-split arrangement was chosen to allocate the treatments i.e.: two inter-row plant spacing and 5 levels of nitrogen which were randomly assigned to the experimental units. Regarding the physiological parameters, measured results revealed that nitrogen and plant inter-row spacing had a significant effect on chlorophyll content and leaf area of the plants. Cultivar Azra7 had large leaf area and higher chlorophyll content under large plant inter-row spacing and 200 $kgNh^{-1}$ as compared to that of Azra3. The yield and the yield component were significantly affected by treatments applied in this experiment. Cultivar Azra7 gave significantly higher yield grain under narrow inter plant spacing and 200$kgNha^{-1}$. Concerning the effect of treatments tested on the content of hydrocyanic acid of two cultivars, the results showed significant difference

Keywords: *Sorghum , Nitrogen, Growth, Yield, hydrocyanic acid.*

Introduction

The reduction of rains leads to a decreased productivity of crops and green cover in the pastures, which negatively affects productivity and reduction in food availability for animal feeding, which leads to the reduction of the quantity of animal protein for human. Sorghum requires less moisture than other cereal crops and is more tolerant to drought prone and of poorly drained soil, making production easier in most agro-ecological zones subject to limited rainfall areas which are unfavorable for most cereals (Maunder,2002). Little works have been conducted in the effect of inter row plant spacing and nitrogen rates on sorghum cultivars in Libya, therefore the objectives of study are to:

1.Determine the best inter-row plant spacing and the optimum nitrogen fertilizer rate for the studied sorghum cultivars to ensure more production quantity and quality of green fodder, grain yield per unit area under the environmental conditions of Libya. 2. Determine the effect of fertilizer on the content of the hydrocyanic acid (HCN) at flowering stage for sorghum cultivars under this study for safe animal feeding.

Sorghum (Sorghum bicolor (L.) Moench), a tropical plant belonging to the Poaceae family is one of the most important cereal crops in the world. Change in row width has significant effects on the spatial distribution of plants. Jacob (2011) Studied the optimum row spacing and plant population for sorghum production. He concluded that 20 cm row spacing produced the greatest amount of fresh biomass. Planting too low population causes increased weed competition (Norsworthy and Oliver, 2002), loss of light interception, and reduces the potential for maximum yield (Edwards and Purcell, 2005). Studies have shown that grain yield significantly differ at different nitrogen levels and grain yield increased at more nitrogen

application rates (Khaliq et al., 2009). In general, the highest forage production of sorghum is observed under rates from 230 to 300 kg N ha^{-1}, with the highest N, P, and K extraction. 150 kg N ha^{-1} is recommended to obtain good sorghum forage production with higher N efficiency levels (Arnall et al., 2013). Increases in leaf photosynthesis rates were observed under higher N levels in grain sorghum hybrids (Cechin, 1998). Plant density strongly affects leaf area index as a consequence light interception and photosynthesis would also be affected (Gan et aI., 2002). Mousavi et al. (2012) observed that the application of N up to 150 kg ha^{-1} increased grain number, grain yield, and harvest index in sorghum. In the study on the effect of different N rates on sorghum yield, Zaongo et al., (1997) found a direct relationship between N and 1000-grain weight. Mahmoud et al., (2013) studied the effect of nitrogen Fertilizer application and plant density on production and quality of sweet sorghum, results revealed that increasing N rate up to 120 kg/fed significantly increased growth traits in terms of leaf area, stalk diameter and stalk length. The production of cyanogenic (hydrocyanic HCN) is one of the major factors limiting the utilization of sorghum fodder due to its toxic effect on feeding livestock (Haskins et al., 1987). (Sher et al., 2012) concluded sorghum forage cultivar JS-2002 with seed rate of 125 kg ha^{-1} without N application produced safe forage for livestock at 50% heading stage under dryland farming conditions. However, variation of rainfall and temperature during crop growth period would affect HCN concentration.

Materials and Methods

The research was conducted on 15 may 2013 in the farm of the glasshouses project of Misurata city. The experimental site was 32016'10.10" N 150 02'49.66" E. The field experiment was split-split plot arrangement in a randomized complete block design with 3 replicates. were used in experiment: Two cultivars (Az3 and Az7) which were introduced from Syria, Also, assigned to the main plots. Whereas the inter row spacing's of 20 and 50 cm were randomly allotted to split plots and the intra row plant spacing was fixed to 50 cm. Each plot dimension was 4.0 by 2.0 m (4 rows). Five fertilizer treatments were chosen (based on the previous studies and the experimental site soil analysis). Fertilizer 75kg $p_2o_5ha^{-1}$ source of phosphors 18% super phosphate, and 75kg k_2o ha^{-1} source potassium sulphate constant for all treatments. Urea (46% N) was used as nitrogen source at a rate of: zero nitrogen per hectare as a control treatment; 100; 150; and 200 kg Nha^{-1} and organic fertilizer (Cow manure) at a rate of 30 ton ha^{-1}. Sown date 16 MAY 2013, four seeds were sown per hill. Ten days after germination the seedlings were thinned leaving two similar seedlings in vigor per hill.

Prior to starting the experiment, soil samples from the experimental site chosen were taken randomly at two depths (0 - 30 and 30 – 60 cm). Sampling was done using a hand probe and samples consisted of 5 individual cores mixed together to form composite sample. the soil was analyzed for pH 8.1-8.2, electric conductivity (EC) 0.77-3.68, Soil texture Sandy silty and silt, Total Ca % 9.3-37.2, Active Ca % -, 14, C% 0.32- 0.32, Organic matter % 0.56- 0.24, total nitrogen%0.52- 0.47 and for manure 0.48%, , C/N ratio 6.15- 6.48, available phosphorus 4 – 1 ppm and for manure 0.22%, exchangeable potash 388- 218 ppm and for manure 0.23%. soil physical properties were also determined. Based on the results obtained from the soil analysis. and the data obtained from the literature the rate of nitrogen that applied in the experiment was determined. Data were collected from middle two rows from each replicate. Eliminating boarder effects, each replicate was separated by two alleys to reduce N fertilizer effects. Results and discussion of the parameters were analyzed using statistical Analysis System (SAS) statistical software. Based on analysis of variance, least significant differences (LSD) was used for treatment means comparison at 0.05 levels and student T-test was used to separate the mean between the varieties tested.

Leaf area (LA) cm2 was measured at the flowering stage, Samples of third leaf from the flag leaf, Individual leaf area was determined by using the following expression given by Sticker (1961) using the following formula: LA = length of Leaf × maximum width of leaf × 0.75. Leaf chlorophyll content (mg/g) A leaf sample was taken from each treatment for chlorophyll content estimation following the methods of Witham et al., (1971) and Bansal et al., (1999). Green fodder Plants were harvested at the beginning when 50% of the plants flowered for fodder. The whole plant weight (kg/plot) was recorded. The fresh weight of the shoot components was determined by using sensitive balance. Weight (g) of 1000 grain count threshed glumeless grain was determined using electronic balance. Grain yield after harvesting the two middle lines from each plot, the panicle was cut and grains from separated panicles were weighted and determined (kg). Hydrocyanic acid (HCN) determination: were determined according to the method followed by Haque and Bradbury (2000) and Bradbury et al., (1999).

Results and Discussion

Leaf Chlorophyll Content: Table (**1**): Effect of inter row plant spacings and nitrogen rates on leaf chlorophyll content (mg/g) of two sorghum cultivars.

Fertilizers	Cultivar Azra3			Cultivar Azra7		
Kgha^{-1}	Spacing 50x50	Spacing 20x50	Mean	Spacing 50x50	Spacing 20x50	Mean
N0=zero	8.86±0.25^{b}	8.16±0.30^{a}	8.51	9.86±0.20^{b}	8.13±0.25^{b}	8.995
N1=100	10.83±0.15^{a}	8.93±0.15^{a}	9.88	12.26±0.35^{a}	9.33±0.25^{b}	10.795
N2=150	11.56±0.20^{a}	9.30±0.10^{a}	10.43	13.26±0.20^{a}	10.23±0.30^{a}	11.745
N3=200	13.20±0.30^{a}	9.70±0.26^{a}	11.45	14.45±0.41^{a}	11.46±0.35^{a}	12.955
Manure	9.26±0.40^{b}	8.23±0.15^{b}	8.745	10.73±0.25^{b}	8.93±0.25^{b}	9.83
Mean	10.74±1.64	8.86±0.64	9.8	12.11±1.73	9.62±1.20	10.865

LSD at 5%, V= 0.0004, S = 0.0001, F = 0.0001, V*S=0.5806 V* F=0.0004, V*S*F= 0.0057

Data are means ± standard deviation . n=3 Means in the same column with similar letters are not significantly different at p<0.5 level of significantly. Where: V=cultivars, S=Spacing, F=Fertilizers, V*S=cultivars*Spacing, V*F= cultivars* Fertilizers, V*S*F= Varieties*Spacing*Fertilizers

Table (1) showed the effect of inter-row plant spacing and nitrogen rate on leaf chlorophyll content of the two cultivars tested. Increasing plant density was accompanied by a decrease in the concentration of leaf chlorophyll content of both cultivars. The general average of chlorophyll content was 10.9 mg/g of leaf fresh weight. The amount of chlorophyll content decreased in narrow planting (200.000 Plant ha^{-1}) as compared with the large planting (80.000 plant ha^{-1}). The chlorophyll content of plant leaves expresses the effectiveness of the leave photosynthesis Ori., et al. (1999). Increasing the nitrogen level increasing the chlorophyll content because it was the main component of the chlorophyll molecule (Martinez and Guiamet, 2004). Increased plant density increases competition for water, light, and chlorophyll-forming nutrients, reducing chlorophyll levels by increasing plant density (Subedi, and Ma., 2005).

Increasing nitrogen rates were accompanied by an increased concentration of chlorophyll. The highest concentration associated with the treatment of 200 kg N ha^{-1} (13.20 mg\g leaf fresh weight) of cultivar Azra3 for large and narrow inter-row plant spacing, whereas organic manure did not exceed any nitrogen rate except the control treatment in both cultivars. The increase in Nitrogen has a role in increasing the efficiency of carbon assimilation by increasing the chlorophyll content of the leaf. Increasing plant density found to increase competition between plant part and net assimilation ratio of the results were similar to that stated by The current results were consistent with that stated by other researchers (Addy et al. 2010) who investigated nitrogen levels with different cultivars. He stated that higher nitrogen rates led to higher leaf chlorophyll index for stay-green sorghum cultivar (i.e., the phenotypes that exhibit delayed senescence) as having higher chlorophyll content during the grain-filling

period and maturity. Genotypic differences in post-anthesis nitrogen uptake can affect leaf senescence patterns and grain yield (Borrell and Hammer, 2000; Borrell et al. 2000a, 2000b). Higher nitrogen levels led to higher leaf chlorophyll index for stay-green sorghum during anthesis (Addy et al., 2010), an important factor in determining not only onset of leaf senescence but also the rate of post-anthesis leaf senescence. Increases in leaf photosynthesis rates were observed under higher nitrogen levels in grain sorghum hybrids (Cechin, 1998). Although leaf senescence in sorghum during grain filling is affected greatly by the water supply/demand balance, it is also affected by the nitrogen supply/demand balance. The potential nitrogen translocation rate from vegetative plant parts to the grain in cereals has been related to the amount of translocate able nitrogen available in these plant parts at anthesis (Jamieson and Semenov, 2000).

Leaf Area: Table (**2**): Effect of inter row plant spacings and nitrogen rate on leaf area of the cultivars tested at flowering stage (cm^2/leaf).

Fertilizers	Cultivar Azra3			Cultivar Azra7		
Kgha^{-1}	Spacing 50x50	Spacing 20x50	Mean	Spacing 50x50	Spacing 20x50	Mean
N0=zero	265.93±4.6^{b}	293.73±6.2^{c}	279.83	346.00±5.0^{c}	311.80±3.9^{a}	328.90
N1=100	330.10±7.3^{a}	312.10±4.3^{b}	321.10	380.73±4.9^{b}	344.06±2.1^{a}	362.40
N2=150	337.80±2.9^{a}	334.06±6.9^{a}	335.93	385.60±4.0^{b}	356.26±6.5^{b}	370.93
N3=200	380.60±4.4^{a}	350.36±4.2^{a}	365.48	430.33±3.0^{a}	365.93±6.1^{b}	398.13
Manure	296.56±6.6^{b}	304.60±3.4^{b}	300.58	354.63±4.5^{c}	337.40±3.7^{b}	346.01
Mean	322.198	308.97	315.584	379.458	343.09	361.274

V= 0.0004, S = 0.0001, F = 0.0001, V*S=0.0001 V* F=0.0009, V*S*F= 0.0001

Table (2) showed the effect of inter-row plant spacing and nitrogen levels applied to the two cultivars tested in this study. The results revealed that there were significant effects of the interaction of inter-row spacings and nitrogen levels on the leaf area at the flowering stage.
Higher plant density was accompanied by a significant increase in the leaf area at the flowering stage in the studied cultivars. Cultivar Azra7 have a large leaf area as compared with cultivar Azra3.
Increasing nitrogen levels was accompanied by an increase in leaf area at flowering stage. The differences between the cultivars was significant at 0.05%. This might be due to the effect of nitrogen and low density as stated by Borras et al., (2003); Borrell et al., (2000). who stated that increasing nitrogen rates enhancing cell division and increasing the width of leaves and vice versa with decreasing nitrogen levels (Cechin, 1998).

Green Fodder: Table (**3**)**:** Effect of inter row plant spacings and nitrogen rates on green fodder (ton ha^{-1}) of the two cultivars tested.

Fertilizers	Cultivar Azra3			Cultivar Azra7		
Kgha^{-1}	Spacing 50x50	Spacing 20x50	Mean	Spacing 50x50	Spacing 20x50	Mean
N0=zero	9.34 ±0.25	13.10 ± 0.10^{c}	11.22	10.16 ± 0.76^{c}	14.70 ±0.26^{c}	12.43
N1=100	14.73±1.71^{b}	28.30 ± 5.11^{a}	21.51	20.16 ± 8.71^{a}	21.90 ±5.28^{b}	21.03
N2=150	13.46±1.15^{b}	19.80 ± 2.69^{b}	16.63	16.30 ±8.87^{b}	21.16 ±1.95^{b}	18.73
N3=200	18.20±2.62^{a}	27.00 ± 1.41^{a}	22.6	15.06±3.66^{b}	27.73±3.53^{a}	21.395
Manure	13.3± 7.24^{b}	15.93 ± 5.85^{c}	14.61	11.43±5.66^{c}	19.10±4.22^{b}	15.265
Mean	13.80	20.82	17.31	14.622	20.918	17.77

LSD at 5% , V= 0.0150 S = 0.0001 F = 0.0001 V*S=0.0361 V* F=0.0693 , V*S*F= 0.4893

Table (3) showed the effect of plant density and nitrogen rates applied to the cultivars under test on the green fodder produced. The result obtained revealed no significant differences between the cultivars due to the interaction between inter-row plant spacing and rates of nitrogen.
Narrow planting produce about 27 ton ha^{-1} under 200 kg N ha^{-1} for both cultivars. The reduced green fodder produced in large inter plant spacing might be due to the reduction in the number of plants per unit area. These results are consistent with the findings of Deghani and Bahrani (2004).

Concerning the amount of nitrogen fertilizer added to produce fodder Poaub et al., (2015) found that 120 kg Nha^{-1} was the optimum amount to produce good yield. Comparing this to our results, it was a bit different. This might be due to deference in soil fertility of site. In general, increasing nitrogen rates result in higher production, was confirmed by the results found by (Kashani and Bahrani et al.1980).

1000-Grain Weight: Table (4): Effect of inter row plant spacings and nitrogen rates on 1000-grain weight of the two cultivars.

Fertilizers	Cultivar Azra3			Cultivar Azra7		
$Kgha^{-1}$	Spacing 50x50	Spacing 20x50	Mean	Spacing 50x50	Spacing 20x50	Mean
N0=zero	18.00± 0.60^{b}	17.03±0.15^{a}	17.515	20.93± 0.30^{b}	18.10±0.26^{b}	19.515
N1=100	19.93± 0.11^{b}	18.33±0.40^{a}	19.13	22.83±0.45^{a}	19.33±0.57^{b}	21.08
N2=150	20.43± 0.58^{b}	19.00±0.10^{a}	19.715	23.66 ±0.35^{a}	19.90 ±0.55^{b}	21.78
N3=200	22.80±0.43^{a}	20.33±0.57^{a}	21.565	24.76± 0.32^{a}	21.16± 0.28^{a}	22.96
Manure	19.23± 0.25^{b}	18.20±0.20^{a}	18.715	21.93± 0.90^{b}	18.80±0.52^{b}	20.365
Mean	20.078	18.578	19.328	22.822	19.458	21.14

LSD at 5% V= 0.0001, S = 0.0001, F = 0.0001,V*S=0.0001, V* F=0.0001, V*S*F= 0.0001

Table (4) showed the effect of plant inter-row spacing and nitrogen rate on the weight of 1000 grain weight. The results revealed a significant interaction effect on the weight of the grains. Increasing the density of the plant leads to competition between the roots on the mineral nutrients, which contributed to a reduction in the weight of the grain. This finding is consistent with the findings of Blum., (1970), Heiniger and Vanderlip.,(1997).

The increase in nitrogen levels increased the weight of 1000 grains in both cultivars. It increased from 19.13 to 21.565 g in cultivar Azra3 with an increase in the nitrogen rate from 100 - 200 kg N ha^{-1}. Organic fertilizer was superior to the control treatment and near the values of treatment 100 kg N ha^{-1}. Cultivar Azra7 was increased from 18.1 to 21.16 g with an increase in the nitrogen rate from zero to 200 kg N ha^{-1}.

Nitrogen increases the plant's ability to increase photosynthesis products, which increases the grain's fullness and thus increases its weight by prolonging the length of green leaves. This finding is consistent with the finding of Baker and Blamey (1985).

The effect of plant densities was non-significant with nitrogen rates in grain weight for both cultivars. The lowest plant density with the highest nitrogen content gave the maximum weight of the grain while the highest plant density was absolved with the lowest nitrogen weight of the grain but no significant differences were recorded at 0.05%.

Our result confirmed the results stated by Ashiono et al., (2005) who carried out an experiment at Kenya Agricultural Research Institute, Nakuru, Kenya and found that 1000 seed weight (22.5g) in dual purpose sorghum was higher with the application of 50 kg N ha^{-1}on deep sandy loam soils than other nitrogen levels (0 kg N ha^{-1}, 20 kg N ha^{-1}, 30 kg N ha^{-1} and 40 kg N ha^{-1}).

Grain Yield: Table (5): Effect of inter row plant spacings and nitrogen rates on grain yield (ton ha^{-1}) of the two cultivars.

Fertilizers	Cultivar Azra3			Cultivar Azra7		
$Kgha^{-1}$	Spacing 50x50	Spacing 20x50	Mean	Spacing 50x50	Spacing 20x50	Mean
N0=zero	0.982±0.02^{b}	1.366±0.02^{c}	1.174	1.479±0.06^{c}	1.955±0.05^{c}	1.717
N1=100	1.948±0.06^{a}	2.177±0.03^{a}	2.0625	2.762±0.06^{b}	3.766±0.06^{a}	3.264
N2=150	2.026±0.04^{a}	2.315±0.10^{a}	2.1705	3.270±0.05^{a}	3.849±0.07^{a}	3.5595
N3=200	2.565±0.05^{a}	3.145±0.14^{a}	2.855	3.599±0.20a	4.897±0.58^{a}	4.248
Manure	1.124±0.01^{b}	1.876 ±0.05^{b}	1.500	2.004±0.16^{b}	2.393±0.10^{b}	2.1985
Mean	1.729	2.1758	1.9524	2.6228	3.372	2.9974

LSD at 5% V= 0.0001, S = 0.0001 F = 0.0001V*S=0.0006 V* F=0.0001V*S*F= 0.0003

Table (5) shows significant differences in grain yield between the varieties, planting distances, fertilization rates and interaction between the varieties and fertilizers.

The increase in plant density was accompanied by a significant increase in total grain yield, which increased significantly from 1.729 to 2.1758- ton ha^{-1} in the cultivar Azra3 for both

large and narrow spacing respectively. This result found to comply with the finding of and Ramshe et al., (1985).

The higher in nitrogen rates was accompanied by a significant increase in the total grain yield of each of the studied cultivars. It increased from 1.174 to 2.855 ton ha^{-1} for the cultivar Azra3, increasing the nitrogen rate from zero to 200 kg N ha^{-1} while the organic fertilizer produced 1.5 ton ha^{-1} it was more than control treatment. Cultivar Azra7 increased from 1.717 to 4.248-ton ha^{-1} by increasing the nitrogen rate from zero to 200 kg ha^{-1} while the organic fertilizer was 2.1985-ton ha^{-1}, which exceeded the control treatment. These results are consistent with that found by Ramshe et al., (1985).

Increasing the density of the plant as well as reducing the number of leaves also leads to competition between the roots on the mineral nutrients, which leads to a reduction in the weight of the grain. This finding is consistent with what researchers have found. (Blum, 1970 and Heiniger and Vanderlip,1997).

Nitrogen fertilizer has the ability to increase photosynthesis products, which helps to fill the grain's and thus increase their weight by prolonging the length of green leaves. This finding is consistent with what other researchers found Baker et al., (1985). The current results confirmed the results stated by George et al., (2014) who observed that sorghum hybrids and inbred lines varied in their response to N fertilizer. As a consequence, there were significant differences in physiological and yield traits among the genotypes tested.

Increases in sorghum grain yield were mainly associated with improving panicle number, grain number per panicle, and grain weight (Buah et al., 2012). Mousavi et al., (2012) observed that application of nitrogen up to 150 kg ha^{-1} increased grain number, grain yield, and harvest index in sorghum. Greater yields and components of yield with increases in nitrogen application were also observed in maize (Zea mays L.) and wheat (Triticum aestivum L.), (Arnall et al. 2013).

HCN Content at Flowering Stage: Table (**6**): Effect of inter row plant spacing and nitrogen rates on HCN content at flowering stage (ppm) of the two cultivars.

Fertilizers	Cultivar Azra3			Cultivar Azra7		
Kgha^{-1}	Spacing 50x50	Spacing 20x50	Mean	Spacing 50x50	Spacing 20x50	Mean
N0=zero	298.33±11.93^{c}	295.66±6.80^{c}	296.99	95.00±3.00^{d}	99.66±2.08c	97.33
N1=100	360.33±10.01^{b}	472.66±6.65^{b}	416.49	107.00±4.58^{c}	141.00±2.64^{a}	124.00
N2=150	404.66±2.51^{a}	580.66±4.04^{a}	492.66	137.66±2.51^{b}	145.33±2.51^{a}	141.49
N3=200	428.33±2.08^{a}	591.00±11.13^{a}	509.66	205.66±0.05^{a}	150.33±0.76^{a}	177.99
Manure	250.33±6.02^{c}	229.00±9.64^{c}	239.66	225.33±6.02^{a}	126.00±3.00^{b}	175.66
Mean	348.396	433.796	391.09	154.13	132.464	143.29

LSD at 5% V= 0.0001, S = 0.0001 F = 0.0001V*S=0.0001 V* F=0.0001V*S*F= 0.0001

The results at a table (6) showed significant differences in the hydrocyanic acid content (HCN) between the two cultivars. The increase in plant density was accompanied by a significant increase in the content of hydrocyanic acid at the flowering stage which increased from 348.4 to 433.8 ppm for cultivar Azra3 for both large and narrow inter raw spacing respectively. Cultivar Azra7 with an average of 143.3 ppm. The increase in nitrogen levels was accompanied by an increase in the content of the hydrocyanic acid at the flowering stage of each studied cultivars. This indicates the contribution of nitrogen fertilizer to cyanide increase. Hydrocyanic acid contents heritable and subjected to modification through selection and breeding, as well as by climate, stage of maturity, stunting of plant, type of soil and fertilizer (Khatri et al., 1997). Nitrogen application is considered essential for growth and regrowth during the growing season. However, a higher level of nitrogen application may increase hydrocyanic acid contents of forage sorghum; ultimately poisoning animals (Aziz-Abdel and Abdel-Gwad, 2008). Optimum plant density and nitrogen applications are essential pre-requisites to utilize the available soil and environmental resources effectively. (Sher et al., 2012) concluded from the results of two years of field experiments that management strategies in combination with genetic variability could produce safe sorghum forage free hydrocyanic acid (HCN).

Conclusions

The cultivar Azra7 was superior to cultivar Azra3 for grain yield and total aboveground biomass. Maximum total leaf chlorophyll content and grain yield were obtained at 200 kg N ha^{-1} and the lowest yield was obtained at 0 kg N ha^{-1}. No strong relationship was found between chlorophyll content and grain yield in the two cultivars studied under the environmental condition of this experiment. Application of 200 kg N ha^{-1} correlated with narrow inter-row plant spacing resulted in an increase in yield of 13 and 48% over 0 kg N ha^{-1}, respectively for the two cultivars viz. Azra7 and Azra3. Similarly, zero kg N ha^{-1} and organic fertilizer used in this study resulted in a decreased amount of leaf chlorophyll content, this reflected the importance of the nitrogen fertilizer in the enhancement of nitrogen in the formation of the chlorophyll pigment which is a major factor that affect the gross photosynthesis that enhance the growth of the plants. Under the current study, we found that the cultivars differ significantly in their content of hydrocyanic acid at the flowering stage, cultivar Azra3 having large amounts of hydrocyanic acid. It is also found that increasing nitrogen rates resulted in increasing content of hydrocyanic acid during the vegetative growth in both cultivars. Therefore, applications of 200 kg of N ha^{-1} is recommended for both cultivars. Conclusively, from the results obtained in this study, we recommend that more investigations should be carried out and introduction of new hybrid lines and genotypes with low hydrocyanic acid content to be tested in Libya in the future and genotypes that can tolerate the prevailing environment conditions e.g. drought and salinity.
summing up from this study we recommend cultivating Azra7 under 200 kg N/ha^{-1} and high plant densities (200000 plant ha^{-1}) for the production of fodder for livestock safety from HCN and grain yield .

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MORPHOLOGICAL AND BIOCHEMICAL DIVERSITY AMONG 'BEYAZ TURFANDA' GRAPE CLONES

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Abstract

Grapevine is an ancient agricultural crop, yet despite the massive advances in cultivation and domestication, many of the commercial varieties that make up the bulk of the international wine and fresh market have been in production since the Roman times. Coruh Valley is rich in terms of grape biodiversity and grape varieties grown in valley stand out with its different taste and thin peel traits due to different climatic condition of the valley. The main grape variety in the valley is 'Beyaz Turfanda' and the vineyards include this variety approximately %95 ratio. The variety has big berry and cluster size. It is observed that diverse 'Beyaz Turfanda' clones in Yusufeli district in Coruh valley has been appeared during long growing period. In this study 10 'Beyaz Turfanda' clones were selected and evaluated according to berry weight (g), cluster size (cm^2), cluster length (cm), cluster width (cm), Soluble Solid Content (SSC), pH, acidity and vitamin C. Results showed diversity for both morphological and biochemical parameters among the 'Beyaz Turfanda' clones. The clones showed cluster size between 195 cm^2 and 253 cm^2.

Keywords: *Grape, diversity, cluster, berry.*

Introduction

Grapevine is one of the oldest cultural plants that have been grown since ancient times throughout world. It is cultivated under a variety of soil and climatic conditions in three distinct agro-climatic zones, namely, tropical, subtropical and mainly temperate regions in different parts of the World (This et al., 2006). In temperate regions leaves of grapevines fall down in winter months but in tropical regions grapevines remain evergreen. The grape genus Vitis comprises three natural groups based on geographical location: North America, Eurasia and Asia. Botanists list from 25 up to 30 species of American origin, about the same number for Asia, but only a single species for Euroasia: *Vitis vinifera*. It is cultivated throughout the world today. In contrast with the American and Asiatic vines, the wild populations of Vinifera in Europe are now almost extinct, occurring in widely separated clumps, often reduced to a few vines in protected localities (Mc Govern et al., 2004). Cultivated grapes, *Vitis vinifera* subsp. *sativa* L., have been derived from wild grapes, *Vitis vinifera* subsp. *sylvestris* Gmel, via natural or artificial hybridization and selection between the seventh and the fourth millennia BC. South Caucasia and west of Hazar Lake, including Anatolia, have a great wild grape potential (Zohary and Hopf, 2000). Turkey is located within the region where grape domestication from the wild ancestor has begun. The country, with richness in local grape varieties around the mountainous regions, has an ancient and conventional viticulture tradition. Tradition grapevine cultivation has commonly been characterized with a large vineyard, a spacious garden (Uzun and Bayir, 2010). One of the principle centers of grape cultivation in Turkey is the Aegean, with seedless sultan's grapes. They are used not for wine or table grapes as in other grape growing regions, but for yellow, golden raisins, with Turkey being the number one exporter in the world with 25% of the market. The grapevine was cultivated in the Mediterranean region for thousands of years although it is a native of Central

Asia. Approximately 5 percent of the grape production is used for wine making in Turkey. The rest, consumed as dried and fresh and there are a number of indigenous wine grapes of Turkey. Nearly all Turkish regions have favorable ecological conditions for viticulture. Only at higher altitudes of Eastern Anatolia and Black Sea Coastal Region (Northern Anatolia) where the rainfall is over 2.000 mm, viticulture has certain limitations (Celik et al., 2008).
Woody crops are propagated by vegetative methods including grafting or cuttings, which enables the desirable qualities of the variety to be perpetuated. However, somatic mutations arise spontaneously and accumulate through successive generations of propagation, resulting in phenotypic and agronomic differences and the creation of new clones, and ultimately new cultivars or trademarked clones. Several important examples are the various sports of Pinot Noir, including Pinot Meunier, Pinot Gris and Pinot Blanc, all of which arose through somatic mutation of Pinot Noir, but which are now classed as varieties in their own right. These example in fact highlight one of the major challenges in understanding the basis of clonal variation, as the mutation exist only in the outer cell layer (L1), that is the sports are chimeric, a mixture of two spatially separate genomes (Myles et al., 2011; Atak et al., 2014).
The aim of this study to compare different 'Beyaz Turfanda' clones grown together in Cevreli village belongs to Yusufeli district in Turkey.

Material and Method

In this study, ten grape clones of 'Beyaz Turfanda' were used. It is a main variety in Yusufeli region in Turkey and dominates in grape production with 95% ratio. The cultivar is mainly used as table grape due to thin peel characteristics. It is also used for molasses production in the region. Comparing with the other grape varieties in Yusufeli region, 'Beyaz Turfanda' seems more resistant to diseases.
The clones were sampled from different locations of Cevreli village of Yusufeli district at the altitude of 960 m in 2018. The grape clusters were harvested in the periods when the berries reached full maturity. The berry samples were picked homogenously and were brought immediately to laboratory for morphological measurements and biochemical analyses. Average berry weight, cluster size, cluster width and cluster length were determined as morphological assessment. Berry weight was determined on 30 random sampled berries per clone by using a digital balance with a sensitivity of 0.001 (Scaltec SPB31). Linear dimensions as length and width of 30 clusters per clone were measured by using digital caliper gauge with a sensitivity of 0.001 mm. For biochemical assessment, SSC (Soluble Solid Content), acidity, pH and vitamin C were determined on 10 randomly taken cluster samples from each clones. SSC of juice were determined by digital refractometer (Kyoto Electronics Manufacturing Co. Ltd., Japan, Model RA-250HE). The acidity, pH and Vitamin C of berries were determined by AOAC methods (AOAC, 1984).

Statistical analysis

The study encompassed four replications for berry and cluster characteristics. In the statistical analyses, Windows SPSS 20 was used and the differences between the means were evaluated by subjecting to ANOVA variance analysis and determined with Duncan multiple comparison test ($p < 0.005$).

Results and Discussion

The variance analysis revealed that there were statistically significant differences among 'Beyaz Turfanda' clones for all morphological and biochemical traits ($p < 0.05$) (Tables 1, 2). Average berry weight, cluster size, cluster width and cluster length were changed between

6.30 g (Y1) and 7.41 g (Y6); 195 cm^2 (Y9) and 253 cm^2 (Y2); 16.67 cm (Y9) and 18.55 cm (Y2); and 10.58 cm (Y9) and 12.50 cm (Y3), respectively (Table 1). Previous studies conducted on different clones of grape varieties showed diversity on berry weight and cluster size and dimensions. For example Dilli et al. (2015) conducted a study on clonal selection of 'Siyah Gemre' grape cultivars and they selected 33 promising 'Siyah Gemre' clones. The clones showed berry and cluster weight between 2.80-4.40 g and 323-552 g. At the and of study, they selected superior 'Siyah Gemre' clones according to a special scoring system for yield, growth and quality parameters. Fidelibus et al. (2006a) studied on Chardonnay grapevine clones in USA and Chardonnay clones showed differences and clone 4 generally had heavier clusters than the other clones, whereas clones 6 and 15 generally had lighter clusters. They stated that differences in cluster weight were mainly due to the number of berries per cluster.

Our results are in agreement to those obtained by Wolpert et al. (1995), who evaluated seven Cabernet Sauvignon clones from California and found significant differences regarding bunch and berry weights among clones. These results agree with those reported by Alonso et al. (2004), who observed morphological variability between clones of the Albariño variety grown in Spain. Burin et al. (2011) reported bunch weight as 113 g and 147 g among 169 and 685 clones of Cabernet Sauvignon grapes in Brazil. Vujovic et al. (2016) compared 11 Merlot clones in terms of average length of grape cluster, number of grape clusters per shoot, number of berries per grape cluster, length of peduncle, length of berry and berry juice yield. They reported that most of the above morphological characteristics differed significantly among the examined clones and the cluster and principal component analyses classified 11 samples into three divergent clusters/groups. Aldo et al. (2016) selected 16 superior clones of cultivar Malbec in Argentina. They showed that sensory and physical-chemical characteristics of clones quite differed from each other.

As shown in Table 2, SSC, pH, acidity and Vitamin C of 'Beyaz Turfanda' clones were found between 11.55% (Y2) and 14.02% (Y9); 3.18 (Y2) and 3.51 (Y7); 1.30% (Y5) and 1.55% (Y2); and 23.45 mg/100 g (Y1) and 28.40 mg/100 g (Y6), respectively.

Fidelibus et al. (2006a) studied on Chardonnay grapevine clones in USA and Chardonnay clones showed biochemical differences. They reported that the berries of clone 4 had higher soluble solids, lower pH, and similar or higher titratable acidity than the berries of other clones. Burin et al. (2011) evaluated two clones, 169 and 685, of Cabernet Sauvignon grapes in Brazil and they showed that these two clones revealed significant differences regarding chemical composition. Zamuz et al. (2007) analyzed different clones of the Albariño variety from the same location and observed a significant difference among clones regarding physical-chemical parameters in must, at harvest, which indicates that classical parameters could be used to differentiate between clones of the same grape variety. Ferrandino and Guidoni (2010), evaluated different clones of the Barbera variety grown in the same location and found significant differences between clones on pH, soluble solids, and total acidity.

Previously some studies conducted on different grape varieties such as Tempranillo, Cavus, Pinot Noir, Merlot, and Cabernet Sauvignon, have shown that, within the same grape variety, different clones can be distinguished by number and weight of clusters, berry size and weight, chemical compositions etc. (Arozarena et al., 2002; Castagnoli et al., 2006; Fidelibus et al., 2006b, 2007; Atak et al., 2014; Ranković-Vasić et al., 2015).

Table 1.Berry weight, cluster size, cluster length and cluster width of 'Beyaz Turfanda' clones

Clones	Berry weight (g)	Cluster size (cm^2)	Cluster length (cm)	Cluster width (cm)
Y1	6.30cd	227b	17.25ab	11.34b
Y2	6.52c	253a	18.55a	12.44ab
Y3	7.01b	237ab	18.41ab	12.27ab
Y4	6.67bc	244ab	18.36ab	12.50a
Y5	7.12ab	204bc	17.11ab	11.06bc
Y6	7.41a	198c	17.03ab	10.95bc
Y7	7.20ab	210bc	18.02ab	11.40b
Y8	7.33ab	201c	17.00ab	11.20bc
Y9	7.30ab	195c	16.67b	10.58c
Y10	7.07b	214bc	18.18ab	11.25bc

Different letters in same colons indicate statistically significant differences ($p<0.05$)

Table 2. Soluble Solid Content, pH, Acidity and Vitamin C of 'Beyaz Turfanda' clones

Clones	Soluble Solid Content (%)	pH	Acidity (%)	Vitamin C (mg/100 g)
Y1	12.68d	3.30ab	1.44ab	23.45i
Y2	11.55f	3.18c	1.55a	25.95e
Y3	13.00cd	3.41ab	1.38bc	25.00g
Y4	12.27e	3.24bc	1.42b	25.60f
Y5	13.04cd	3.33ab	1.30c	24.00h
Y6	13.37bc	3.20bc	1.51ab	28.40a
Y7	13.60b	3.51a	1.53ab	27.10c
Y8	13.10c	3.30bc	1.44ab	27.70b
Y9	14.02a	3.27b	1.47ab	28.05ab
Y10	13.55b	3.41ab	1.56a	26.80d

Different letters in same colons indicate statistically significant differences ($p<0.05$)

Conclusion

The present results showed that 'Beyaz Turfanda' clones differed from each other in terms of some important morphological and biochemical characteristics. Our results agree with those observed in other studies, which indicate that it is possible to differentiate between clones of the same grape variety. On a closer inspection, this work could have effects on viticulture in Yusufeli, by providing for the first time an instrument of identity, as well as identification of 'Beyaz Turfanda' clones. In this context, therefore, we believe that this effort has not only a scientific importance for the improvement of grapevine cultivation, but also a social and socio-economic impact for viticulture in Yusufeli.

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CHARACTERIZATION OF LOCAL SOUR CHERRIES FROM ARTVIN PROVINCE IN TURKEY

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Abstract

The production and consumption of cherries have increased recently due to consumer awareness of their health benefits. Coruh valley is located in Northeastern Anatolia in Turkey and it is famous area in the world in terms of plant biodiversity. In this study we sampled ten sour cherry genotypes from Ardanuc town belonging to Artvin province and some phenotypic traits such as harvest date, fruit mass, flesh ratio, fruit peel color and biochemical characteristics such as SSC (Soluble Solid Content), titratable acidity, antioxidant capacity, total anthocyanin, total phenolic and vitamin C content were determined. Sour cherry genotypes were harvested between 23 June and 10 July, but the majority of the genotypes were harvested between 25 and 30 June. The genotypes had average fruit mass between 2.34 g and 3.42 g. The majority of genotypes had red fruit skin color and followed by dark red fruit skin color. Fruit flesh ratio of local sour cherry genotypes ranged from 84.70 to 90.20%. Local sour cherry genotypes were found to be rich source of bioactive content and total phenolic content were in range of 256 to 328 mg gallic acid equivalent per 100 g. Total anthocyanin content also differed significantly among genotypes which were found between 114 to 189 mg cyanidin-3-glucosylrutinoside equivalent per 100 g fresh samples.

Keywords: *Local fruits, Sour cherry, Characterization, Germplasm*.

Introduction

The production and consumption of cherries has increased recently due to consumer awareness of their health benefits, since they are rich in polyphenolics (namely anthocyanins and hydroxycinnamic acids). Global sweet cherry production increased over the last 16 years from 1.90 to 2.32 million tons, with Turkey, USA, Iran, as the main producers. Sour cherry production has been static during the same period at around 1.1–1.3 million tons concentrated in Europe, with the Turkey, Poland and Russian Federation are the predominant producer (FAO, 2018). The predominant cultivar in the US (Michigan) was 'Montmorency', in Europe (Croatia, Hungary and Serbia, respectively) 'Marasca', 'Balaton' and 'Oblacinska' and in Turkey 'Kütahya' (Blando and Oomah, 2019). Sour cherry (*Prunus cerasus* L.) is an allotetraploid species, originating from a natural hybridization between sweet cherry (*P. avium* L.) and ground cherry (*P. fruticosa* Pall.) (Iezzoni, 2008). The distribution of European sour cherry ranges from the Mediterranean islands to northern Russia, and, within the range there is a wide diversity of different plant habitats and fruit characters (Beaver et al., 1995). Sour cherries (*P. cerasus* L.) are most popular as fruit crop and in fruit industry in Turkey. In Turkish it is called Vişne. It is also called Višně in Czechoslovakia, wiśnia in Poland, vyshnya in Ukranian, vishnja in Albanian, višna in Bulgaria, vişine in Romania, višanja in Bosnia, and Croatia, vísino in Greece, and vişnya in Russia. Sour cherries have a unique tartness, giving them an added dimension of cherry flavour and unlike their sweet counterparts, they come in a variety of different shades, making them extra special. Sour cherries are also hold their authentic cherry flavour throughout a high-heat cooking process, making them the ideal choice for baked goods or jams. Since that tart flavour demands a bit of sugar, it makes for a

perfect match in cakes and pies (Papp et al., 2010). In Turkish cuisine, regular cherries are only eaten out of hand and enjoyed only as fruit, but seldom used in desserts, dishes or preserving. There are only a few exceptions; the so-called white cherries that are actually yellow of pinkish are pickled in the Black Sea region, especially in Giresun. The city is believed to be the birthplace of cherries, its ancient name "Cerasus" getting its name from cherries or cherries getting its name from the city. The growing of sour cherry in Turkey plays an important economic role. The fruit is grown on all agricultural regions, and the annual harvest ranges from 180 to 200 thousand tonnes. Coruh valley located Northeastern Anatolia in Turkey and it is famous area in the world in terms of plant biodiversity. Local sour cherry trees and shrubs could be found each points of the valley and sour cherry fruits contribute livelihoods of the local communities peoples. Seed propagated sour cherries also an important part of biodiversity of the valley. Recent breeding programs have focused on the natural biodiversity and local landraces present in a specific region. The characterization of germplasm collection of autochthonous cultivars for nutraceutical traits can be important to select new genotypes high in organoleptic quality and antioxidants content (Albano et al., 2014; Blando and Oomah, 2019). Numerous studies have been done on physical and chemical characteristics in sour cherry cultivars (Pedisic et al., 2007; Khorshidi and Savarynejad, 2010; Papp et al., 2010), while studies on seed propagated sour cherries are rare. Also, no detailed study concerning physical and chemical properties of seed propagated sour cherry has been performed up to now. This aim of this study to determine morphological and biochemical potential of 20 native seed propagated sour cherry genotypes grown in northeastern part of Turkey.

Material and Methods

The material (10 seed propagated genotypes) were harvested in Ardanuc district located in Northeastern part of Turkey in 2017 year. A number of morphological measurements and biochemical analysis were done on 10 genotypes. Along with 10 genotypes, the standard sour cherry cultivar Kutahya was included study. We used 40 fruits per genotype with four replications for measurements. Fruit weight was measured by electronic balances. Fruit flesh ratio and fruit peel color were determined on these samples as morphological parameters. For biochemical analysis vitamin C, SSC and acidity were determined. SSC was determined by refractometer. Acidity in juice and Ascorbic acid (Vitamin C) of samples was quantified with the reflectometer set of Merck Co (Merck RQflex). Total phenolic content of sour cherries were measured with Folin–Ciocalteu method (Magalhães et al., 2010) and expressed as milligram gallic acid equivalent per 100 g pitted fresh sour cherry fruit (mg GAE/100 g). Total anthocyanin content was measured spectrophotometrically (Krawczyk and Petri, 1992). Total anthocyanin content was calculated as cyanidin-3-glucosylrutinoside equivalent per 100 g fresh sour cherry fruit. FRAP (Ferric reducing antioxidant power) assay was used for antioxidant capacity analysis. The FRAP was expressed as mmol per 100 g of Trolox equivalent on the basis of the fresh weight of fruits (Benzie and Strain, 1996).
For analysis of variance, the obtained data were used for means calculation. Duncan multiple range tests were performed at the significant level of $p<0.05$.

Results and Discussion

Harvest dates of 10 seed propagated sour cherry genotypes quite variable ranging from 23 June to 10 July. The majority of the genotypes harvested earlier than standard cultivar Kutahya and only one genotype (A4) harvested same time with cv. Kutahya (Table 1). Previous studies are also indicated harvest time differences among sour cherry genotypes and

cultivars. In particular unnamed genotypes shows great variability on harvest time. Mika et al. (2011) used four sour cherry cultivars such as 'Debreceni Botermo' ('Debreczyn'), 'Nefris', 'English Morello' and 'Sokówka Serocka' (No. 29) in their experimental and they reported that the beginning of fruit ripening in the cultivar 'Debreceni Botermo' came, depending on the year, between the 10-20 June. Soon after, the cultivar Nefris began to ripen, and about 7 days later the 'Sokówka Serocka' and 'English Morello' reached to ripening. Harvesting of 'English Morello' sour cherries was possible until 10 July. Khadivi-Khub (2014) used 24 sour cherry genotypes in Iran and found that harvest dates were changed between 24-30 JuIy among genotypes. In Aegean region of Turkey, 43 unnamed sour cherry genotypes were selected and harvest date of them were found to be between 13 June and 25 June (Onal, 2002). In another study conducted in eastern Anatolia in Turkey, sour cherry genotypes were harvested between 18 July and 21 July (Tekintas et al., 1991). In Germany, harvest date were found between mid July and end of July for sour cherry cultivars (Schuster and Wolfram, 2005). A wide variation on harvest time for genotypes is an important element and makes them promising genotypes for future sour cherry breeding.

The 10 sour cherry genotypes exhibited fruit mass between 2.34-3.42 g. Previously studies on both local and cultivated sour cherry genotypes showed variation on fruit mass. Khadivi-Khub (2014) used 24 sour cherry genotypes in Iran and found that fruit mass were between 2.25 and 3.36 g. Also, Papp et al. (2010) reported range of 3.40–7.17 g for fruit mass of Hungarian sour cherries. It should be noted that rootstock and crop load might also influence fruit weight (Goncalves et al. 2006). In Turkey Tekintas et al. (1991) found fruit mass of five sour cherry genotypes between 1.91 and 3.63 g that indicate similarities with our results. Tanis (2010) sampled 31 different cv. Kutahya types from Northeastern Anatolia (Gumushane region) and determined fruit mass between 1.9 and 5.3 g. Therefore, large fruited genotypes may be considered as perspective parents and for cultivation. Fruit mass and characteristics depend on cultivar, maturation stage, agricultural practices and environmental conditions (Gonçalves et al., 2006).

As indicated in Table 1, 10 sour cherry genotypes had diverse fruit peel color. Among genotypes, 3 genotypes had dark red and 7 genotypes had red fruit peel color. In terms of fruit juice color, 9 genotypes had dark red, 9 genotypes had red and 2 genotypes had light red fruit juice color (Table 1). Khadivi-Khub (2014) used 24 sour cherry genotypes in Iran and found that fruit peel color were in general red to black. In Turkey Onal (2002) reported light red, red and dark red fruit peel color in sour cherry selections. Color intensity is the most commonly used indicator of maturity, depending on the cultivar. In sour cherries, color is determined mostly by the concentration and distribution of red pigments, the anthocyanins in the skin. Changes in skin color are positively correlated with anthocyanin accumulation during ripening (Blando and Oomah, 2019).

As shown in Table 1, fruit flesh ratio of 10 seed propagated sour cherry genotypes were in range of 84.70-90.20%. Tanis (2010) determined fruit flesh ratio between 86.4-93.5% in sour cherries in Turkey. Literature survey revealed that sour cherry fruits had flesh ratio between 85.0-93.0% (Popa et al., 2011; Yilmaz and Gokmen, 2013; Chaovanalikit and Wrolstad, 2004). In Iran a large variations on fruit weight/ stone weight in all sour cherry germplasm were detected (Khadivi-Khub, 2014).

Soluble Solid Content (SSC), titratable acidity, vitamin C, total phenolic content, total anthocyanin content and total antioxidant capacity and of the sour cherry genotypes and cv. Kutahya are shown in Table 2. All biochemical parameters of genotypes significantly differed each other at 0.05 statistical level indicating diversity among genotypes (Table 2).

SSC content of genotypes ranged from 10.80% to 14.70% and cv. Kutahya had 13.76% SSC content (Table 2). Wojdylo et al. (2014) found that sour cherries had SSC content in the range of 12.90 to 25.15%. Tekintas et al. (1991) reported SSC content of five sour cherry genotypes

between 15.0-20.0% in Eastern Turkey. Tanis (2010) studied on 31 different cv. Kutahya types from Northeastern Anatolia (Gumushane region) and revealed SSC content between 10.5-17.1%. SSC and acidity are two important internal factors that affect quality and strongly influence its taste and market value of fruits. SSC content of fruit was affected by many factors including genotype, soil, climate conditions and sampling periods (Ercisli et al., 2012)
Titratable acidity of 10 sour cherry genotypes is given in Table 2. Titratable acidity quite variable among genotypes varied from 1.40 to 2.04%. The cv. Kutahya titratable acidity value as 1.80% (Table 2). Tanis (2010) used 31 different cv. Kutahya types from Northeastern Anatolia (Gumushane region) and determined titratable acidity between 1.1-2.2%, which in agreement with our result. Many internal and external factors including genotype, soil, climate conditions and sampling periods affects acidity of fruit and acidity is an important factor affecting fleshy fruit taste (Ercisli et al., 2012).
The genotypes had vitamin C content between 9.40 and 14.00 mg/100 g FW (Table 2). The Kutahya cultivar had vitamin C as 11.35 mg/100 g FW (Table 2). Sour cherries are known low vitamin C crop. Wojdylo et al. (2014) characterized a number of sour cherry germplasm in Poland and reported vitamin C between 5.54-22.18 mg/100 g. The difference among studies could be effects of environmental conditions, plant genotypes, analyze methods, ripening time, cultivation conditions etc.
Total phenolic content is one of the important bioactive content of fruits. Table 3 shows total phenolic content of genotypes and cv. Kutahya. Total phenolic content were found to be between 256 and 328 mg GAE/100 g FW among genotypes. The cultivar cv. Kutahya had 303 mg GAE/100 g FW total phenolic content. Four genotypes had higher TPC than cv. Kutahya and the rest of genotypes had similar TPC with cv. Kutahya highlighting importance of seed propagated genotypes for breeding perspective. The results are also showed that significant variation on total phenolic content among seed propagated materials. Previously sour cherry fruits reported variable TPC (from 78 to 500 mg GAE/100 g FW) (Bonerz et al., 2007; Dragović-Uzelac et al., 2007).
Total anthocyanin content of sour cherry genotypes are shown in Table 2. The genotypes exhibited total anthocyanin content between 114-119 mg cyanidin-3-glucosylrutinoside equivalents/100 g FW (Table 2). A few genotypes had higher anthocyanin content than cv. Kutahya while the majority of genotypes had lower values than cv. Kutahya. In Denmark Khoo et al. (2011) reported anthocyanin content among 34 sour cherry cultivars from 21 to 272 mg malvidin-3-glucoside equivalents/100 g of fresh fruit weight. In Hungary, anthocyanin content of sour cherries were found between 100-220 mg/100 g FW (Veres et al., 2008). These differences in total anthocyanin content showed that the genotypes used, growing region and the harvest period might have an impact on plant growth and metabolite concentration (Premier, 2002).
Total antioxidant capacity of seed propagated sour cherry genotypes and cv. Kutahya shown in Table 2. FRAP value of genotypes were between 7.16 and 12.81 mmol Trolox/100 g fresh sour cherry fruit samples. The cultivar Kutahya had 9.15 mmol Trolox/100 g antioxidant capacity indicating lower values than majority of sour cherry genotypes (Table 3). In literature, sour cherries have been investigated extensively for the total antioxidant capacity and all studies indicated strong genotype dependent antioxidant capacity in sour cherry fruits (Seeram et al., 2001; Blando et al., 2004; Bonerz et al., 2007; Pedisić et al., 2007; Veres et al., 2008; Khoo et al., 2011).
Results from this study showed that seed propagated rich bioactive content and could be promising breeding material for the development of varieties with high nutraceutical value under low-input management.

Table 1. Morphological characteristics of seed propagated sour cherry genotypes and cv. Kutahya

Genotypes	Harvest date	Fruit mass (g)	Fruit peel color	Flesh ratio (%)
A1	3 July	2.67c	Dark red	84.90
A2	26 June	3.33bc	Red	85.05
A3	23 June	2.34bc	Red	87.30
A4	5 July	3.42b	Dark red	84.70
A5	29 June	3.28bc	Red	85.25
A6	28 June	3.15bc	Red	85.55
A7	25 June	2.95bc	Red	90.20
A8	30 June	2.88bc	Dark red	86.26
A9	28 June	3.07bc	Red	88.42
A10	30 June	3.03bc	Red	86.40
Kutahya	05 July	4.47a	Dark red	85.40c

Means within a column followed by the same letter are not significantly different at $p<0.05$

Table 2. Bioctive content seed propagated sour cherry genotypes and cv. Kutahya

Genotypes	Total phenolic (mg GAE/100 g FW)	Total anthocyanin (mg/100 g FW)	FRAP (mmol Trolox/100 g FW)	Vitamin C (mg/100 g FW)	SSC (%)	T. acidity (%)
A1	295bc	189a	11.45d	9.40c	13.10ab	1.80a
A2	315ab	153d	12.64bc	13.65ab	10.65cd	1.55b
A3	282c	114f	10.80de	12.15ab	9.90e	1.40bc
A4	256d	180ab	9.40ef	11.25b	12.90b	1.70ab
A5	267cd	145de	9.87ef	9.70bc	10.40d	1.64ab
A6	320ab	166c	13.30b	13.80ab	10.80cd	1.60b
A7	328a	134e	14.20a	14.00a	11.60c	1.58b
A8	307b	173ab	12.10c	10.50bc	10.25d	1.30c
A9	290bc	125ef	11.13de	9.90bc	11.30cd	1.60b
A10	275cd	150de	10.07e	10.05bc	11.20cd	1.55b
Kutahya	303b	177b	9.15f	11.35bc	13.76a	1.78a

Means within a column followed by the same letter are not significantly different at $p<0.05$

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COMPARISON OF DROUGHT TOLERANCE IN DURUM WHEAT USING VARIOUS STRESS SELECTION INDICES

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Abstract

This study was performed to evaluate the ability of several selection indices to identify tolerant genotypes under a drought stress conditions. Fourteen durum wheat genotypes were evaluated under both drought stress and non-stress environments using twelve drought tolerance indices. Results showed that genotypes G3 and G4 were the highest yield non-stress condition, while genotype G8 displayed the highest performance under stress condition. The significant and positive correlation of YP with GMP, MP, STI, K_1STI, YP and HM indices indicated that these indices were more effective in identifying high yielding genotypes under non-stress condition while the positive association of YS with K_2STI indicated that this index was more effective in identifying high yielding genotypes under stress condition. Genotypes performed differently to drought stress, which justifies screening durum wheat for both yield and drought tolerance. Therefore, STI-based indices (STI, K_1STI and K_2STI) can discriminate drought tolerant genotypes with high grain yield under both non-stress and stress conditions. Finally, the genotype G3 besides genotype G8 were identified to be the most favorable genotypes and recommended for cultivation.

Keywords: *durum wheat, principle component analysis, tolerance indices*

Introduction

Grain yield can be evaluated in terms of different yield component traits, some of them can assume more important than the others, depending upon the time and intensity of abiotic stresses and their temporal development. In the Mediterranean regions, most rain falls during the autumn and winter, and water deficit occurs in the spring, resulting in the moderate drought stress for rainfed durum wheat around anthesis and throughout grain filling period (Sabaghnia et al., 2012a; Karimizadeh et al., 2012). This stress may lead to a loss in grain yield; particularly severe deficit at anthesis stage has serious effects on durum wheat grain yield, reducing numbers of spike and spikelet and decreases the fertility of spikelets. Loss of grain yield is the main concern of durum wheat breeders and they emphasize on yield performance under drought stress conditions (Motzo and Giunta, 2007; Sabaghnia et al., 2012b). Breeding for tolerance to drought stress is complicated by the lack of proper screening methods and the inability to create repeatable water drought conditions where large populations can be evaluated efficiently (Sabaghnia et al., 2013).

To differentiate drought tolerant genotypes, several selection indices have been suggested on the basis of a relationship between non-stress and stress conditions. These indices provide a measure of drought based on loss of yield under drought stress conditions in comparison to non-stress conditions. Fischer and Maurer (1978) proposed the stress susceptibility index, and Rosielle and Hamblin (1981) defined stress tolerance and mean productivity for evaluation of genotypes for drought tolerance. The stress susceptibility index is yield performance of a genotype under stress condition as a function of the yield performance without stress, the

stress tolerance is the difference in yield between the stress and non-stress conditions, and the mean productivity is the average yield in both conditions. Bouslama and Schapaugh (1984) suggested yield stability index and Lin and Binns (1988) used the superiority index as estimates of genotype adaptability over a range of environments. The superiority index is the mean square of the distance of the yield performance of a genotype from the maximum observed yield of all genotypes at stress or non-stress conditions. Fernandez (1992) also has been suggested stress tolerance index, geometric mean productivity and harmonic mean for screening breeding materials for stress. Gavuzzi et al. (1997) and Sadiki (2006) proposed yield index and relative reduction to screening drought tolerant genotypes, respectively.
However, the objectives of this investigation were to (i) identify drought tolerant durum wheat genotype under drought stress, (ii) determine the efficiency of screening methods to classify genotypes into tolerant/sensitive and (iii) study interrelationships among the screening indices.

Materials and methods

In this investigation, 14 durum wheat genotypes including 13 new improved genotypes from ICARDA (International Center for Agricultural Research in the Dry Areas) and one checks cultivar from Iran (Dehdasht) were studied under dry-land condition. Each plot was 5 × 6 m, rows with a 20 cm row space and total plot size was 6 m^2. The experiments were conducted a randomized complete block design with four replications under non-stress and stress (rainfed conditions with twice irrigation) conditions. Plants were fertilized with nitrogen at the rate of 50 kg ha^{-1} urea and phosphorus at the rate of 120 kg ha^{-1} ammonium phosphate and proper agronomic management practices were adopted throughout the growing season to ensure good crop growth. After physiological maturity, plots were harvested and grain yield was adjusted to 12.5% seed moisture content.
Various drought tolerance indices were calculated as; the stress susceptibility index (SSI) of Fischer and Maurer (1978):

$$SSI = [1-(Ys/Yp)]/[1-(\bar{Y}s/\bar{Y}p)]$$

where: Ys, yield performance in stress conditions; Yp, yield performance in non-stress condition; $\bar{Y}s$, average of yield performance of all genotypes in stress conditions; $\bar{Y}p$, average of yield performance of all genotypes yield in non-stress conditions. The superiority index (PI) of Lin and Binns (1988) was computed as:

$$Pi = \sum_{j=1}^{n} \frac{(X_{ij} - M_j)^2}{2n}$$

where n is the number of conditions, Xij the seed yield of ith genotype in the jth condition and Mj is the yield of the genotype with maximum yield at condition j. The mean productivity (MP) and tolerance (TOL) of Rosielle and Hamblin (1981) were calculated as:

$$MP = (Ys + Yp)/2$$

$$TOL = (Yp - Ys)$$

The yield stability index (YSI) of Bouslama and Schapaugh (1984) was calculated as:

$$YSI = Ys/Yp$$

The stress tolerance index (STI), geometric mean productivity (GMP) and harmonic mean (HM) of Fernandez (1992) are computed as:

$$STI = (Yp \times Ys)/Yp^2$$

$$GMP = \sqrt{(Ys + Yp)}$$

$$HM = [2(Yp \times Ys)]/(Yp + Ys)$$

The yield index (YI) was calculated according to (Gavuzzi et al., 1997):

$$YI = Ys / \overline{Y}s$$

The STI-based indices consist on K_1STI and K_2STI were assessed as (Naderi et al., 1999):

$$K_1STI = [(Yp)^2 / (\overline{Y}_p)^2]STI$$

$$K_2STI = [(Ys)^2 / (\overline{Y}s)^2]STI$$

The relative reduction (RR) was computed based on Sadiki's (2006) formula:

$$RR = (Yp - Ys) / Yp$$

Genotypes means were compared by the least significant difference method. Phenotypic linear correlation coefficients were calculated for all possible comparisons of drought tolerance indices using the Pearson correlation coefficient.

Results and discussion

Genotypes G3 and G4 were found the highest yield non-stress condition with 4032 and 4025 kg ha^{-1} grain yield performance respectively, while genotype G8 displayed the highest performance (2702 kg ha^{-1}) under stress condition (Table 1). The low yield performance genotype was G7 (3028 kg ha^{-1}) under non-stressed condition and genotype were G2 (2089 kg ha^{-1}) under stressed condition (Table 1). It is interesting that, some genotypes including G1, G3, G6, G8 and G14 had high performances in both stressed and non-stressed conditions and their differences with the best genotypes of both conditions are not significant statistically. Also, the other remained durum wheat genotypes were identified as semi-tolerance or semi-sensitive to drought stress.

Table 1. Mean comparison for yield performances and twelve drought tolerance indices for 14 durum wheat genotypes

	YP		YS		SSI		MP		TOL		STI		GMP	
G1	3838	BAC†	2413	BDAC	1.188	BA	3125	BA	1426	BA	0.00049	BA	3042	BA
G2	3090	E	2089	E	0.834	BDC	2589	F	1001	BDC	0.00040	F	2538	E
G3	4032	A	2573	BA	1.217	BA	3302	A	1460	BA	0.00052	A	3212	A
G4	4025	A	2327	BDEC	1.415	A	3176	BA	1698	A	0.00050	BA	3056	BA
G5	3154	DE	2356	BDEC	0.666	DC	2755	FDE	799	DC	0.00043	FDE	2720	EDC
G6	3835	BAC	2428	BDAC	1.172	BA	3131	BA	1407	BA	0.00049	BA	3051	BA
G7	3028	E	2268	DEC	0.634	D	2648	FE	760	D	0.00041	FE	2612	E
G8	3773	BAC	2702	A	0.893	BDC	3238	A	1071	BDC	0.00051	A	3191	A
G9	3531	BDC	2212	DE	1.100	BAC	2872	FBDEC	1320	BAC	0.00045	FBDEC	2790	BEDC
G10	3502	DEC	2514	BAC	0.824	BDC	3008	BDAC	988	BDC	0.00047	BDAC	2964	BAC
G11	3475	DEC	2347	BDEC	0.940	BDC	2911	BDEC	1128	BDC	0.00045	BDEC	2849	BDC
G12	3509	DEC	2142	DE	1.139	BA	2826	FDEC	1367	BA	0.00044	FDEC	2732	EDC
G13	3768	BAC	2392	BDC	1.147	BA	3080	BAC	1376	BA	0.00048	BAC	3000	BAC
G14	3538	BDAC	2539	BAC	0.833	BDC	3038	BDAC	999	BDC	0.00047	BDAC	2989	BAC

	HM		YI		PI		YSI		RR		K_1STI		K_2STI	
G1	2961	BA	1.014	BDAC	283543	BA	0.628	DC	0.372	BA	0.00057	BAC	0.00051	BDAC
G2	2488	E	0.878	E	787584	BCD	0.679	BDAC	0.321	BDAC	0.00030	E	0.00031	E
G3	3125	A	1.081	BA	189563	BCD	0.644	BDAC	0.356	BDAC	0.00066	A	0.00061	BA
G4	2941	BA	0.978	BDEC	275117	ECD	0.583	D	0.417	A	0.00063	BA	0.00047	BDEC
G5	2685	EDC	0.990	BDEC	642243	ECD	0.757	A	0.243	D	0.00035	DE	0.00043	DEC
G6	2973	BA	1.021	BDAC	260954	ECD	0.633	DC	0.367	BA	0.00056	BAC	0.00051	BDAC
G7	2576	ED	0.953	DEC	751499	A	0.753	BA	0.247	DC	0.00030	E	0.00039	DE
G8	3146	A	1.136	A	200681	E	0.716	BAC	0.284	BDC	0.00057	BAC	0.00066	A
G9	2712	BEDC	0.930	DE	491318	ECD	0.631	DC	0.369	BA	0.00044	DEC	0.00039	DE
G10	2920	BAC	1.057	BAC	367944	BA	0.723	BAC	0.277	BDC	0.00046	BDEC	0.00053	BDAC
G11	2789	BDC	0.987	BDEC	451162	ED	0.680	BDAC	0.320	BDAC	0.00043	DEC	0.00045	BDEC
G12	2643	EDC	0.901	DE	546804	A	0.620	DC	0.380	BA	0.00043	DEC	0.00036	DE
G13	2923	BAC	1.006	BDC	313541	E	0.638	BDC	0.362	BAC	0.00054	BAC	0.00049	BDC
G14	2941	BAC	1.067	BAC	458934	BC	0.719	BAC	0.281	BDC	0.00050	BDAC	0.00060	BAC

†Mean values of the same category followed by different letters are significant at $p \leq 0.05$ level.

Based on the SSI, genotype G4 displayed the lowest amount of SSI while according to MP, genotypes G3 and G8 were found drought tolerance genotypes (Table 1). According to the TOL, genotype G4 and based on the STI, genotypes G3 and G8 were identified as the most drought tolerance genotypes (Table 1). Based on the SSI, genotype G4 displayed the lowest amount of SSI while according to GMP, genotypes G3 and G8 were found drought tolerance genotypes while genotypes G2 and G7 were found drought sensitive genotypes (Table 1). Regarding, yield performance in non-stress and stress condition and SSI, STI, MP, TOL and STI indices, genotypes G1, G3, G6 and G13 were the favorable genotypes. The other remained genotypes were detected as the semi-tolerance or semi-sensitive to drought stress. Genotypes G3 and G8 were identified as most favorable and tolerant genotypes based on HM and YI while genotypes G7 and G12 were found as most tolerant genotypes based on PI (Table 1). Mevlut and Sait (2011) indicated that genotypes with high STI values usually have high difference in yield in two different humidity conditions and also, reported relatively similar ranks for the genotypes by GMP and MP as well as STI index, which suggests that these indices are equal for screening drought tolerant genotypes. According to YSI, RR, K_1STI and K_2STI indices, genotypes G5, G4, G3, and G8 were the most tolerant genotypes, respectively (Table 1). Sabaghnia and Janmohammadi (2014) reported that STI-related indices (K_1STI and K_2STI) are convenient parameters for selecting high yielding genotypes in both stress and non-stress environments whereas relative decrease is observed in yield performance.

The associations among different drought tolerance indices are graphically indicated in a plot of two first principal components (PC1 and PC2) analysis (Fig. 1). The first and second components justified 93.6% of the variations among criteria (58.4 and 35.2% for PC1 and PC2, respectively). The PC1 mainly distinguishes the PI and YSI indices from the other remained indices and the PC2 distinguishes the RR, SSI, YP, K_1STI and TOL indices from the indices which related to each other based on the PC1 scores (Fig. 1). One of the interesting interpretations of this plot is that the cosine of the angle between the vectors of indices approximates the simple correlation coefficient between them. The cosine of the angles does relatively translate into correlation coefficients, since the plot of principal components analysis does explain most of the variation in a data set. Therefore, it could be concluded that the GMP, MP, STI, K_1STI, YP and HM indices are positively associated with each other as group 1 and had no or weak positive association with group 2 (RR, SSI and TOL) as well as group 3 (YI, YS and K_2STI). The association of group 2 with group 3 is relatively near zero (Fig. 1). Also, negative associations were seen between group 1 with PI; and between group 2 with YSI.

There is general agreement that new improved high yielding genotypes are more adapted to favorable growing conditions, while old cultivars have more stable performance under different stress conditions such as drought stress. It was interesting to note positive correlation between STI-based indices and specially K_1STI and YP indicating that K_1STI was positively correlated with non-stressed yield. This finding suggested that some traits that contribute to yield potential may act to increase tolerance to stress and that selection for both K_1STI and YP may counteract each other. Some studies showed that GMP and STI indices are preferred in late drought condition for selecting the most favorable genotypes (Blum, 1996; Akcura et al., 2011) while some other investigation indicated that PI and MP indices are preferred for selecting the most tolerant genotypes (Sio-Se Mardeh et al., 2006; Khalili et al., 2012). Also, we found that K_2STI and YS are correlated to each other and so is preferred for selecting the most tolerant genotypes in drought stress conditions. Relatively, such similar conclusion is reported by Fernandez (1992), Mohammadi et al. (2010) and Sabaghnia and Janmohammadi (2014), which mentioned that priority of STI and K_2STI parameters under level of high to

moderate stress. Akcura et al. (2011) reported STI was able to differentiate genotypes belong to genotypes with high yield performance in both conditions, from the others.

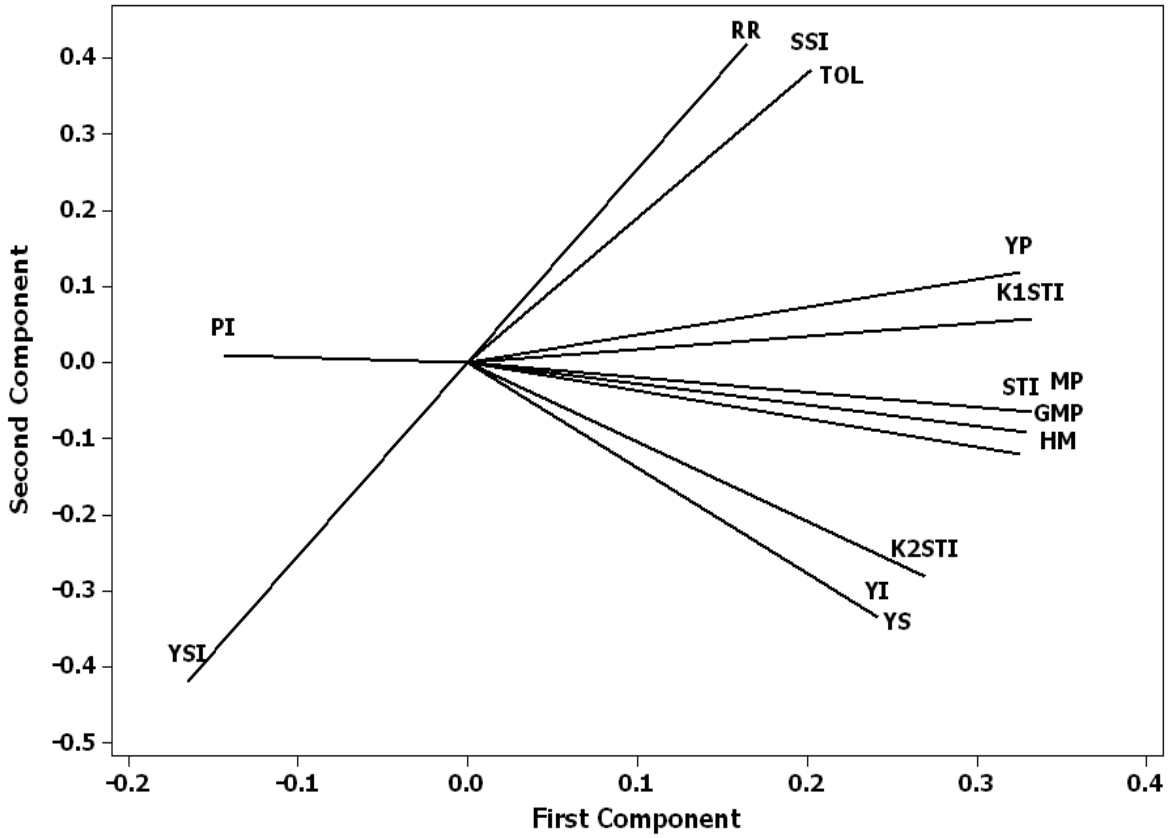


Fig. 1. Tow-way plot based on first two principal component axes (PC1 and PC2) drought tolerance indices in durum wheat genotypes.

Conclusions

In conclusion, according to principal component analysis, the GMP, MP, STI, K_1STI, YP and HM indices indicated positive correlation with YP, while K_2STI showed positive correlation with YS, therefore, STI-based indices (STI, K_1STI and K_2STI) can discriminate drought tolerant genotypes with high grain yield under both non-stress and stress conditions. The genotype G3 (4032 kg ha^{-1} at non-stress and 2573 kg ha^{-1} at stress conditions) besides genotype G8 (3773 kg ha^{-1} at non-stress and 2702 kg ha^{-1} at stress conditions) were found to be the most favorable genotypes and are thus recommended for future recommendation in arid and semi-arid areas of Iran.

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EVALUATION OF MORPHOLOGICAL AND PHYTOCHEMICAL TRAITS AND PHYLOGENETICS RELATIONSHIPS OF SUMMER SAVORY (*SATUREJA HORTENSIS* L.) ACCESSIONS

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Abstract

Iran is one of the most important centers of savory variation and has been distributed in different region from khorasan to west Azerbaijan. Summer savory contains thymol and carvacrol that are used in food and pharmaceutical industries as antioxidant and antimicrobial compounds. In this research, morphological traits diversity in Iranian and foreign countries accessions of summer savory were evaluated. The features of accessions were significantly different and Plant height was the highest in Bulgaria and Uzbekistan accessions (71.31 and 65.14 cm respectively). As expected, Bulgaria accession had the most plant dry weight too (2.53 g per plant). The leaf length varied between 6.84 cm (in Bulgaria accession) to 3.26 cm (in Isfahan accession) and the Leaf width ranged between 1.19 and 0.24 cm (in Ardabil and Isfahan accessions). The highest correlation coefficient was observed between plant fresh weight and dry weight (r= 0.99). There were positive relationships between plant heights and leaf width (r= 0.46), internode length (r= 0.56), main branch number (r= 0.39), branch number (r= 0.47), inflorescence length (r= 0.46), plant fresh (r= 0.57) and dry weight (r= 0.47), petal diameter (r= 0.29) and stem length (r= 0.46). The findings of this research can be used to sketching efficient breeding programs for this species.

Keywords: *Accession, Diversity, Morphological traits, Phytochemical characteristic.*

Introduction

The use of various plant species as flavoring herbs and preserving matter to amend the nutritional properties and medicine compounds have been widely used among different nations since ancient times. Recently natural essential oils of medicinal plants showed scientific interests to make the requirements of health and safety in food and medicine industries (Geetha and Chakravarthula, 2018). Thus the use of homogeneous cultivars with distinguished and improved properties was the prerequisite for the industrial production (Franz, 2000). Existence of genetic diversity among plant accessions originated from different areas can be a reliable opportunity for selection of desirable traits in order to new cultivars production (García-Díaz et al., 2020). Genetic diversity occurs for adaptation of plants to environmental condition and purpose of plant breeding is recognition of favorable accessions among various accessions (Herison et al., 2018)

Satureja hortensis L. is a medicinal plant from the Lamiaceae family having antioxidant, antispasmodic, antimicrobial and antidiarrheal effects and provided an important role in pharmaceutical industry (Semerdjieva et al., 2020). Recently, the antimicrobial and antioxidant properties of herbal medicine were more considered in food and drug science and *S. hortensis* is a rich source of them (Feyzioglu and Tornuk, 2016). The raw material of this species was applied for preservation of food and production of plant derived drugs (Mozafari et al., 2018). Essential oil of *S. hortensis* has a lot of carvacrol that act as antimicrobial agents

(Mazarei and Rafati, 2019) and used for animal production and veterinary medicine (Mozafari et al., 2018). The *S. hortensis* methanol extract is containing rosmarinic acid as an ester of caffeic acid and mostly is responsible for antioxidative, therapeutic properties and anti-inflammatory activity (Nunes et al., 2017). Previously was reported morphological and phytochemical diversity in *S. hortensis* (Hadian et al., 2010). The investigation of morphological diversity represents useful information about species relationships and can be used in plant breeding for indicating new cultivar with promised efficiency (Khadivi-Khub et al., 2012). Hadian et al. (2011) studied eight Iranian populations of *Satureja khuzistanica* based on morphological properties of leaf, flower and essential oil content. Also it was suggested that The Abdanan and Kaver accessions were the best. Phenotypic diversity among Iranian landraces of *S. hortensis* previously was reported by Hadian et al. (2010) Given that hasn't been done a comprehensive study on global potential of diversity among *S. hortensis* accessions yet, therefore the present study was performed to investigation of morphological and phytochemical variability in Asian and European accessions. The accessions having the best features will be detected in this research as well as can be used as initial materials in breeding programs.

Material and Methods

Seeds of 120 samples of *S. hortensis* were collected from different Asian and European accession. Iranian accessions were collected from local field and other countries accessions were gathered from the Institute of Plant Genetics and Crop Research in Germany. The accessions were planted, in Ardabil city's research field (longitude, latitude, and altitude of Ardabil were 48°30′13″, 38°24′94″ and 1333 respectively), in the same condition to variation was determined independent from other agents. The distance between the samples was 400–600 m. Soil of the field was drained and fertilized by manure as well as regularly irrigated to maintain the moisture of soil. During the growing season weeds eliminated manually also any pest or disease was not observed during the experiment. Some morphological and phytochemical properties of accessions were investigated in the flowering step. The traits measured in this study were about different parts of plants, especially principal properties that influencing bioactive characters of plants. The measured traits are listed in Table 1. The *S. hortensis* seeds planted in the field in a randomized complete block design (RCBD) with three replications. The residuals were tested for normality by Kolmogorov–Smirnov normality test using SPSS software. The means were compared using Duncan's multiple range tests at a 5% level of significance. Correlation coefficient for important traits was done based on the Pearson method.

Results and Discussion

The results of mean comparison of morphological traits explained in Table 2. Plant height was the highest in Bulgaria and Uzbekistan accessions (71.31 and 65.14 cm respectively) and lower in Czech, Poland, Rasht and Italy accessions. As expected, Bulgaria accession had the most plant dry weight too (2.53 g per plant) and followed by Uzbekistan (2.53 g per plant), while flower yield showed promise performance in Bulgaria accession (5.32 mg). The most diver features were flower number and leaf fresh weight. Khadivi-Khub et al. (2014) reported that the coefficient of variation ranged between 11.56% (for sepal length) and 120.1 (for stem weight). Previously Hadian et al. (2010) showed among 30 Iranian *S. hortensis* accessions, Tabriz accession had maximum plant height (64.1 g) while in the present study plant height of Bulgaria and Uzbekistan and some other accessions were more than Tabriz accession. The leaf length varied between 6.84 cm (in Bulgaria accession) to 3.26 cm (in Isfahan accession).

The Leaf width ranged between 1.19 and 0.24 cm (in Ardabil and Isfahan accessions). Leaf and plant dimension variability of some studied accessions of *S. hortensis* was shown in Fig. 2. Hadian et al. (2010) reported leaf length varied between 5.05 cm in Tabriz to 1.47 cm in Ahvaz accessions and leaf width varied between 0.71 and 0.28 cm in Tabriz and Urmieh accessions, respectively. In the present study the maximum internode length (5.04 cm) was observed in Bulgaria and the minimum amount obtained in Czech accession. The main branch number and branch number were the highest in Bulgaria (28.76 and 12.97 respectively) while Rasht and Mazandaran showed the lowest main branch number and branch number (9.7 and 1.21 respectively).

The leaf fresh and dry weight was maximum (1.92 and 0.3 g) in Ardabil accession and were minimum in Karaj accession (0.39 and 0.06 g respectively). The Inflorescence length varied between 2.13 cm (in Hungary accession) to 0.73 cm (in Syria accession). The Sepal and petal diameter ranged between 1.16 to 0.23 cm and 1.58 to 0.2 cm, respectively. The plant fresh and dry weight were highest in Bulgaria (23.34 g and 2.53 g respectively) while Kermanshah showed the lowest plant fresh and dry weight (3.93 g and 0.28 g respectively). Shabankare et al. (2015) reported among studied populations of *Teucrium polium* L. ,a member of *lamiaceae*, fresh weight of plants varied between 45.5 g (in Sepidan) and 66.6 g (in Zibashahr). The peduncle length varied between 0.33 cm (in Italy) and 1.4 cm (in Mazandaran). The maximum bract length and width were observed in Mazandaran (2.63 cm) and Ardabil (0.78) respectively while the minimum amount belonged to Germany (0.46 cm) and Hungary (0.1 cm) respectively. The Mazandaran accession showed the earliest flowering (67.93 days after seed germination) and Italy was the longest (115.4 day after seed germination).

Table 1. Descriptive statistics for morphological traits of *S. hortensis* accessions.

Character	Abbreviation	Unit	Mean
Plant height	PHL	cm	36.82
Leaf length	LEL	cm	0.45
Leaf width	LEW	cm	3.02
Internode length	ITL	cm	14.29
Main branch number	MBN	-	4.32
Branch number	BNU	-	0.76
Leaf fresh weight	LFW	g	1.38
Inflorescence length	INL	cm	0.5
Sepal diameter	SED	cm	7.3
Plant fresh weight	PFW	g	0.94
Plant dry weight	PDW	g	0.13
Leaf dry weight	LDW	g	0.8
Peduncle length	PEL	cm	74.32
Day to flowering	DFL	day	0.9
Bract length	BRL	cm	0.34
Bract width	BRW	cm	0.72
Petal diameter	PED	cm	21.35
Stem length	STL	cm	0.31
Stem diameter	STD	cm	4.49
Stamen number	STN	-	0.87
Petal length	PLE	cm	0.29
Shoot color	SHC	Code	4.09
Branch density	BRD	Code	3.38
Leaf density	LED	Code	5.35
Growth habit	GRH	Code	3.74
Leaf upper surface color	LUC	Code	3.97
Leaf lower surface color	LLC	Code	3.64
Seed color	SEO	Code	4.43
Seed length	SEL	cm	3.028
Seed width	SEW	cm	2.37
Flower length	FLL	cm	1.37
Flower width	FLW	cm	22.91
Flower fresh weight	FFW	g	2.3
Flower dry weight	FDW	g	24.35
Leaf area	LAR	Cm^2	3.51
Internode number	INU	-	46.5
Flower number	FLN	-	2.96
Petal color	PEC	Code	2.44
Sepal color	SEC	Code	2.04

Bract color	BRC	Code	267.9
Seed shape	SSH	-	3.73
Bract density	BDE	Code	4.15
Day to germination	DGE	day	4.57
Day to 50% flowering	DTF	day	81.22
Day to seed	DTS	day	106.72

Table 2. Mean comparison of morphological and phytochemical traits in *S. hortensis* accessions.

Region	LEL	LEW	ITL	MBN	BNU	LFW (g)	INL	SED	PFW
Bulgaria	6.84 ^{a}	0.86 ^{ab}	5.04 ^{a}	28.76 ^{a}	12.97 ^{a}	0.77 ^{bc}	1.76 ^{abc}	0.53 ^{bc}	23.24 ^{a}
Germany	3.96 ^{c}	0.34 ^{c}	2.33 ^{cde}	14.16 ^{b}	3.07 ^{b}	0.91 ^{bc}	1.75 $^{a-d}$	0.56 ^{bc}	11.64 ^{bc}
Czech	4.16 ^{c}	0.36 ^{c}	1.83 ^{e}	13.26 ^{b}	5.16 ^{b}	0.65 ^{bc}	1.17 ^{etg}	0.4 ^{bc}	5.65 ^{c}
Georgia	4.33 ^{c}	0.4 ^{c}	2.5 $^{b-e}$	13.83 ^{b}	5.42 ^{b}	0.76 ^{bc}	1.15 ^{etg}	0.4 ^{bc}	10.86 ^{bc}
Syria	3.76 ^{c}	0.26 ^{c}	1.91 ^{e}	13.23 ^{b}	3.56 ^{b}	0.86 ^{bc}	0.73 ^{g}	0.63 ^{bc}	8.51 ^{c}
Hungary	4.12 ^{c}	0.43 ^{c}	3.16 $^{a-e}$	25.94 ^{a}	9.96 ^{a}	0.59 ^{bc}	2.13 ^{a}	0.43 ^{bc}	8.63 ^{c}
Karaj	3.72 ^{c}	0.39 ^{c}	4.02 $^{a-d}$	13.9 ^{b}	4.07 ^{b}	0.39 ^{c}	1.62 $^{a-e}$	0.45 ^{bc}	9.56 ^{bc}
Poland	4.4 ^{c}	0.51 ^{bc}	2.75 $^{b-e}$	11.93 ^{b}	2.55 ^{b}	0.75 ^{bc}	1.16 ^{efg}	0.56 ^{bc}	6.02 ^{c}
Rasht	3.91 ^{c}	0.28 ^{c}	2.01 ^{cd}	9.7 ^{b}	2.26 ^{b}	0.68 ^{bc}	1.96 ^{ab}	0.23 ^{c}	6.03 ^{c}
Italy	4.15 ^{c}	0.28 ^{c}	2.52 $^{b-e}$	10.58 ^{b}	1.02 ^{b}	0.8 ^{bc}	1.05 ^{fg}	0.3 ^{bc}	5.85 ^{c}
Greece	5.54 ^{b}	0.34 ^{c}	4.08 ^{abc}	13.16 ^{b}	3.16 ^{b}	0.4 ^{c}	1.5 $^{b-f}$	0.46 ^{bc}	4.49 ^{c}
Uzbekistan	3.83 ^{c}	0.48 ^{bc}	4.45 ^{ab}	10.05 ^{b}	2.55 ^{b}	0.72 ^{bc}	2.03 $^{-ab}$	0.47 ^{bc}	17.4 ^{ab}
Zanjan	3.89 ^{c}	0.56 ^{bc}	2.16 ^{cde}	12.02 ^{b}	1.23 ^{b}	0.7 ^{bc}	1.01 ^{fg}	1.16 ^{a}	4.31 ^{c}
Kashmar	3.49 ^{c}	0.31 ^{c}	3.08 $^{a-e}$	10.6 ^{b}	2.77 ^{b}	0.73 ^{bc}	1.37 $^{c-f}$	0.63 ^{bc}	4.17 ^{c}
Kermanshah	3.9 ^{c}	0.33 ^{c}	2.73 $^{b-e}$	13.07 ^{b}	1.33 ^{b}	0.88 ^{bc}	1.16 ^{efg}	0.63 ^{bc}	3.93 ^{c}
Tabriz	3.44 ^{c}	1.04 ^{a}	2.83 $^{b-e}$	14.16 ^{b}	3.73 ^{b}	0.83 ^{bc}	1.83 ^{abc}	0.73 ^{b}	4.99 ^{c}
Ardabil	4.14 ^{c}	1.19 ^{a}	3.01 $^{a-e}$	11.54 ^{b}	2.44 ^{b}	1.92 ^{a}	1.16 ^{efg}	0.73 ^{b}	4.06 ^{c}
Mazandaran	3.61 ^{c}	0.4 ^{c}	3.04 $^{a-e}$	15.31 ^{b}	1.21 ^{b}	0.78 ^{bc}	1.56 $^{a-f}$	0.36 ^{bc}	6.9 ^{c}
Kerman	4.09 ^{c}	0.3 ^{c}	3.16 $^{a-e}$	11.85 ^{b}	2.02 ^{b}	1.05 ^{b}	1.04 ^{fg}	0.43 ^{bc}	8.55 ^{c}
Isfahan	3.26 ^{c}	0.24 ^{c}	3.01 $^{a-e}$	15.02 ^{b}	2.06 ^{b}	1.09 ^{b}	1.2 $^{d-g}$	0.4 ^{bc}	6.38 ^{c}

Region	LDW	PEL	DFL	BRL	BRW	PED	STL	STD	PLE
Bulgaria	0.11 ^{bc}	0.4 ^{e}	91.07 ^{bc}	0.6 ^{c}	0.36 ^{bc}	0.39 ^{cd}	27.66 ^{bc}	0.18 ^{b}	0.75 ^{bcd}
Germany	0.14 ^{bc}	0.96 $^{a-e}$	71.2 ^{cd}	0.46 ^{c}	0.3 ^{bc}	0.55 ^{cd}	25.2 ^{bcd}	0.21 ^{b}	0.46 ^{d}
Czech	0.08 ^{bc}	0.56 $^{b-e}$	76.83 ^{bcd}	0.66 ^{c}	0.18 ^{bc}	0.46 ^{cd}	18.33 ^{bcd}	0.31 ^{b}	1.18 ^{ab}
Georgia	0.1 ^{bc}	0.98 $^{a-e}$	81.58 ^{bcd}	0.56 ^{c}	0.19 ^{bc}	0.49 ^{cd}	25.08 ^{bcd}	0.43 ^{ab}	0.7 ^{bcd}
Syria	0.14 ^{bc}	0.86 $^{a-e}$	91.67 ^{b}	0.93 ^{bc}	0.23 ^{bc}	0.41 ^{cd}	26.06 ^{bc}	0.43 ^{ab}	0.73 ^{bcd}
Hungary	0.09 ^{bc}	0.43 $^{d-e}$	80.99 ^{bcd}	0.49 ^{c}	0.1 ^{c}	0.23 ^{d}	26.66 ^{bc}	0.73 ^{ab}	0.43 ^{d}
Karaj	0.06 ^{c}	0.76 $^{a-e}$	76.83 ^{bcd}	0.37 ^{c}	0.2 ^{bc}	0.62 ^{cd}	28.03 ^{b}	0.34 ^{ab}	0.48 ^{d}
Poland	0.14 ^{bc}	0.5 ^{cde}	74.46 ^{bcd}	1.88 ^{ab}	0.28 ^{bc}	0.63 ^{cd}	17.33 ^{bcd}	0.15 ^{b}	0.53 ^{cd}
Rasht	0.11 ^{bc}	0.54 $^{b-e}$	76.83 ^{bcd}	1.02 ^{bc}	0.28 ^{bc}	0.46 ^{cd}	14.16 ^{cd}	0.21 ^{b}	0.55 ^{cd}
Italy	0.12 ^{bc}	0.33 ^{e}	115.4 ^{a}	1.18 ^{bc}	0.07 ^{c}	0.26 ^{d}	12.3 ^{d}	0.13 ^{b}	0.7 ^{bcd}
Greece	0.07 ^{c}	0.7 $^{a-e}$	83.06 ^{bcd}	0.93 ^{bc}	0.13 ^{c}	0.86 ^{bc}	17.66 ^{bcd}	0.3 ^{b}	0.9 $^{a-d}$
Uzbekistan	0.11 ^{bc}	1.36 ^{abc}	89.59 ^{bc}	0.86 ^{c}	0.19 ^{bc}	1.58 ^{a}	45.66 $^{-a}$	0.93 ^{a}	1.06 ^{abc}
Zanjan	0.12 ^{bc}	0.66 $^{b-e}$	74.76 ^{bcd}	1.06 ^{bc}	0.46 ^{abc}	0.67 ^{cd}	21.1 ^{bcd}	0.21 ^{b}	1.33 ^{a}
Kashmar	0.12 ^{bc}	1.56 ^{a}	73.87 ^{bcd}	0.52 ^{c}	0.41 ^{abc}	0.47 ^{cd}	20.09 ^{bcd}	0.23 ^{b}	0.96 $^{a-d}$
Kermanshah	0.14 ^{bc}	0.83 $^{a-e}$	74.46 ^{bcd}	0.88 ^{c}	0.16 ^{bc}	0.66 ^{cd}	17.66 ^{bcd}	0.33 ^{b}	1.13 ^{ab}
Tabriz	0.14 ^{bc}	1.3 $^{a-d}$	81.28 ^{bcd}	0.93 ^{bc}	0.23 ^{bc}	0.76 ^{c}	25.1 ^{bcd}	0.29 ^{b}	1.13 ^{ab}
Ardabil	0.3 ^{a}	0.9 $^{a-e}$	76.99 ^{bcd}	0.95 ^{bc}	0.78 ^{a}	0.62 ^{cd}	19.05 ^{bcd}	0.23 ^{b}	0.76 ^{bcd}
Mazandaran	0.12 ^{bc}	1.4 ^{ab}	67.93 ^{d}	2.63 ^{a}	0.56 ^{ab}	1.26 ^{ab}	20.2 ^{bcd}	0.16 ^{b}	0.7 ^{bcd}
Kerman	0.17 ^{b}	1.02 $^{a-e}$	80.69 ^{bcd}	1.05 ^{bc}	0.28 ^{bc}	0.43 ^{cd}	20.41 ^{bcd}	0.23 ^{b}	0.93 $^{a-d}$
Isfahan	0.14 ^{bc}	1.16 $^{a-e}$	86.9 ^{bcd}	0.96 ^{bc}	0.3 ^{bc}	0.2 ^{d}	16.02 ^{bcd}	0.2 ^{b}	0.7 ^{bcd}

Region	SEL	SEW	FLL	FLW	FFW	LAR	INU	FLN	DTF
Bulgaria	5.61 ^{a}	3.66 ^{b}	3.41 ^{b}	0.44 ^{e}	26.34 ^{a}	37.36 ^{a}	10.98 ^{a}	560.99 ^{bc}	102.33 ^{b}
Germany	1.66 ^{et}	2.03 ^{cd}	2.29 ^{c}	1.08 ^{ae}	22.69 ^{a}	23.5 ^{bc}	6.88 ^{cd}	520.44 ^{bd}	80.05 ^{bc}
Czech	1.94 ^{et}	2.08 ^{cd}	1.83 ^{c}	0.63 ^{be}	7.11 ^{b}	25.97 ^{bc}	5.75 ^{cd}	371.74 ^{bd}	86.33 ^{bc}
Georgia	2.01 ^{et}	1.5 ^{d}	1.61 ^{c}	1.10 ^{ae}	13.66 ^{b}	27.97 ^{ab}	6.8 ^{cd}	506.92 ^{bd}	91.67 ^{bc}
Syria	4.66 ^{abc}	2.49 ^{c}	1.91 ^{c}	0.97 ^{ae}	10.71 ^{b}	21.18 ^{c}	6.99 ^{cd}	527.2 ^{bc}	103.04 ^{b}
Hungary	3.03 ^{ct}	2.21 ^{cd}	2.08 ^{c}	0.48 $^{d-e}$	10.87 ^{b}	25.41 ^{bc}	7.62 ^{bd}	540.71 ^{bc}	91.11 ^{bc}
Karaj	3.37 ^{bcd}	1.71 ^{d}	1.61 ^{c}	0.86 ^{ae}	12.03 ^{b}	20.7 ^{c}	7.93 ^{bc}	567.75 ^{b}	81.02 ^{bc}
Poland	4.73 ^{abc}	2.33 ^{cd}	2.5 ^{bc}	0.56 ^{ce}	7.58 ^{b}	28.77 ^{ab}	5.85 ^{cd}	351.46 ^{bd}	83.67 ^{bc}
Rasht	2.21 ^{et}	1.13 ^{d}	1.6 ^{c}	0.61 ^{be}	7.59 ^{b}	22.94 ^{bc}	6.25 ^{cd}	297.39 ^{cd}	84.33 ^{bc}
Italy	4.74 ^{abc}	2.33 ^{cd}	1.81 ^{c}	0.37 ^{e}	7.36 ^{b}	31.28 ^{ab}	5.5 ^{d}	256.84 ^{d}	129.67 ^{a}
Greece	3.02 ^{def}	1.66 ^{d}	2.07 ^{c}	0.79 ^{ae}	5.65 ^{b}	30.49 ^{ab}	9.36 ^{ab}	364.98 ^{bd}	93.33 ^{bc}
Uzbekistan	5.38 $^{-a}$	3.66 ^{b}	3.33 ^{c}	1.53 ^{ac}	26.14 ^{a}	30.53 ^{ab}	11.03 ^{a}	925.97 ^{a}	100.67 ^{b}
Zanjan	3.33 $^{b-f}$	2.04 ^{cd}	1.77 ^{c}	0.74 ^{be}	5.42 ^{b}	22.7 ^{bc}	7.28 ^{bd}	425.81 ^{bd}	84.04 ^{bc}
Kashmar	3.31 $^{b-f}$	1.75 ^{cd}	1.75 ^{c}	1.75 ^{a}	5.24 ^{b}	17.94 ^{c}	7.69 ^{bd}	405.53 ^{bd}	83.05 ^{bc}
Kermanshah	2.01 ^{et}	1.82 ^{cd}	1.96 ^{c}	0.93 ^{ae}	4.95 ^{b}	11.56 ^{c}	7.42 ^{bd}	358.22 ^{bd}	83.67 ^{bc}
Tabriz	5.01 ^{ab}	1.27 ^{d}	1.44 ^{c}	1.45 ^{ad}	6.28 ^{b}	22.86 ^{bc}	7.57 ^{bd}	506.92 ^{bd}	91.33 ^{bc}
Ardabil	5.06 ^{ab}	5.66 ^{a}	6 ^{a}	1.01 ^{ae}	5.1 ^{b}	25.69 ^{bc}	6.95 ^{cd}	451.21 ^{bd}	86.33 ^{bc}
Mazandaran	2.77 ^{et}	2.33 ^{cd}	1.66 ^{c}	1.57 ^{ab}	8.68 ^{b}	19.3 ^{c}	6.83 ^{cd}	419.05 ^{bd}	76.33 ^{c}
Kerman	1.5 ^{f}	1.64 ^{d}	1.4 ^{c}	1.13 ^{ae}	10.76 ^{b}	25.13 ^{bc}	7.57 ^{bd}	416.99 ^{bd}	90.67 ^{bc}
Isfahan	4.05 $^{a-d}$	5.07 ^{a}	5.33 ^{a}	1.3 ^{ad}	8.02 ^{b}	11.83 ^{c}	6.42 ^{cd}	324.43 ^{bd}	97.67 ^{bc}

Means within a column followed by the same letter are not significantly different at ($p<0.05$).

Table 2 (continued)

Character	Abbreviation	Code and frequency (%)			
		1	3	5	7
Shoot color	Shc	Light-green (12)	Green (54)	Dark-green (35)	Reddish green (19)
Branch density	Brd	Low (33)	Intermediate (60)	High (23)	Very high (4)
Leaf density	Led	Low (27)	Intermediate (45)	High (40)	Very high (8)
growth habit	Grh	Drooping (2)	Spreading (73)	Spreading to upright (30)	Upright (15)
Leaf upper surface color	Luc	Light-green (37)	Green (51)	Dark-green (23)	Reddish green (9)
Leaf lower surface color	Llc	Light-green (57)	Green (41)	Dark-green (20)	Reddish green (2)
Seed color	Seo	Light-brown (19)	Brown (38)	Dark-brown (44)	Black (19)
Petal color	Pec	White (23)	Purple (49)	Dark-Purple (22)	Pink (26)
Sepal color	Sec	Light-green (22)	Green (75)	Dark-green (23)	-
Bract color	Brc	Light-green (38)	Green (53)	Dark-green (29)	-
Seed shape	Ssh	Medium-elliptic(44)	Elliptic (31)	Circular (45)	-
Bract density	Bde	Low (33)	Intermediate (60)	High (23)	Very high (4)

The correlation coefficients of morphological traits were shown in Table 3. There were positive relationships between plant heights and leaf width (r= 0.46), internode length (r= 0.56), main branch number (r= 0.39), branch number (r= 0.47), inflorescence length (r= 0.46), plant fresh (r= 0.57) and dry weight (r= 0.47), petal diameter (r= 0.29) and stem length (r= 0.46). The plant height had a positive correlation with most important morphological traits. Previously the similar results were reported by Mohebodini et al. (2018) about the investigation of Garden Cress Landraces diversity. This result agrees with Hadian et al. (2014) study, which suggested there is a positive correlation plant height and fresh and dry weight (r= 0.96 and 0.45 respectively). That is in agreement with Mehdi et al. (2017) results. These achievements can be used in breeding programs for cultivar production.

Table 3.Correlation coefficients among morphological traits of *S. hortensis* accessions.

	PHL	LEW	ITL	MBN	BNU	LFW	INL	SED	PFW	PDW	LDW	PEL	DFL	BRL	BRW	PED	STL
PHL	1																
LEW	0.46**	1															
ITL	0.56**	0.21	1														
MBN	0.39**	0.03	0.2	1													
BNU	0.47**	0.17	0.33	0.81**	1												
LFW	-0.11	0.3**	-0.12	-0.17	-0.22	1											
INL	0.46**	0.01	0.32	0.24	0.28	-0.23**	1										
SED	0.08	0.25	-0.13	-0.13	-0.13	0.26**	-0.03	1									
PFW	0.57**	0.15	0.41**	0.37**	0.49**	0.05	0.19	-0.06	1								
PDW	0.47**	0.11	0.34**	0.4**	0.54**	-0.04	0.14	-0.06	0.99**	1							
LDW	-0.09	0.32**	-0.05	-0.2	-0.25	0.86**	-0.21	0.14	-0.03	-0.04	1						
PEL	-0.007	-0.06	0.02	-0.31	-0.35**	0.2	0.04	0.26**	-0.16	-0.17	0.08	1					
DFL	0.16	-0.16	0.06	-0.06	-0.08	0.006	0.08	0.05	0.02	0.04	-0.01	0.002	1				
BRL	-0.14	-0.05	-0.06	-0.19	-0.26	0.18	-0.11	0.06	-0.04	-0.03	0.2*	0.25**	-0.06	1			
BRW	0.06	0.15	-0.12	-0.03	-0.03	0.25	0.02	0.2*	-0.1	-0.14	0.22*	0.07	-0.18	0.17	1		
PED	0.29**	0.11	0.11	-0.3**	-0.33**	0.01	0.21	0.07	-0.01	-0.04	0.14	0.29**	-0.15	0.29**	0.2*	1	
STL	0.46**	0.02	0.35**	0.22	0.27	-0.09	0.16	-0.14	0.5**	0.4**	-0.007	0.09	-0.06	-0.15	-0.16	0.21*	1

Conclusions

In this study agro-morphological features of *S. hortensis* accessions that originated from Iran and some Asian and European countries were evaluated. The different accessions were grown in uniform climatic and cultivation conditions, thus variation in plant characteristics is related to genetic factors. The observed diversity gives to plant breeder ability for the indication of the cultivars with desirable traits to use in the pharmaceutical industries. Essential oil due to antioxidant and antimicrobial activity and being safer than chemical counterparts is considerable and this study represented a novel suggestion for the use of *S. hortensis* global potentials to use in plant breeding programs.

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AN INSIGHT INTO THE CHEMICAL COMPOSITION AND BIOMEDICAL POTENTIAL OF THE GRAPESEED OIL-COLD PRESSED

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Abstract

This mini review paper aimed to put some light into the chemistry and medicinal uses of the grapeseed oil-cold pressed possessing numerous biologically active compounds of relevance both for health and nutrition. Without a doubt, its production has been fully justified both in terms of economy and ecology – in brief, the grape seeds are broadly considered as a by-product of the fruit processing. This vegetable oil (making 13-18% of the seed's content), enriched in the unsaturated fatty acids essential for humans, is widely recognised for its lively colour, aroma and taste. Linoleic (over 70%) and oleic (about 15%) acids are predominant fatty acid compounds, followed by linolenic acid (approximately 1%). In comparison, the content of the saturated fatty acids usually reaches around 10%. Tocopherols (α - 10 mg/100 g; β and γ - 15 mg/100 g) and tocotrienols (mostly γ) also contribute to the oil's composition. While the most common sterol is β-sitosterol (67-70%), campesterol, stigmasterol and avenasterol may be present at a smaller scale. Finally, polyphenolic compounds (or, simply, polyphenolics) are worth mentioning herein, too. Such an unique chemistry does provide a lot health benefits, in particular, towards heart disease. More precisely, the risk of cardiovascular disease can be reduced up to 55% by the oil's proper (adequate) use. Additionally, the cancer patients are likely to profit on its enormous biopotential with a clear stress on the antioxidant effects.

Keywords: *Grapeseed oil, Secondary metabolites, Health value.*

Introduction

Being substantial ingredients of the regular human diet, oils and fats provide great amount of energy followed by important chemicals such as fatty acids and vitamins. In addition to this, they are well implicated into a number of processes contributing to normal physiological functions. As indispensable ingredients in the preparation of meals, oils and fats need to be of rather high quality, in order to ensure the proper growth and development of an organism. Their use mostly depends on the age, lifestyle, occupation, customs and eating habits. Generally speaking, fats may cover up to 25-30% of daily average energy requirement (Dimić, 2005). According to the recommendations of the Food and Agriculture Organisation (FAO) of the United Nations, the unsaturated fatty acids should make 15-20%, the saturated ones up to 10%, while the polyunsaturated fatty acids in the range 6-11%.

Grapeseed oil is semi-dry, greenish-golden yellow in colour. After a period of the prolonged standing, the colour becomes brownish. Its sensory properties are pleasant and similar to those of olive oil, but with a less pronounced wine-fruit aroma and mild notes of grapes and walnuts (Morin, 1996; Dimić, 2005; Matthäus, 2008; Rubio *et al.*, 2009). This oil is very stable at high temperatures, has a high smoke point (200-230°C) and slightly increased viscosity, if used for frying.

Table 1. The recommended intake of fat (expressed as % of total energy intake).

Country	Total Fat	Saturated Fatty Acids	Polyunsaturated Fatty Acids	Monounsaturated Fatty Acids
Canada	< 30	<10	0.5-3.0	–
USA	< 30	<10	<10	–
UK	< 30	<10	7.5	–
Australia	30	10	7-10	>10
New Zealand	30	12	8	20
Japan	20-25	6-8	6-8	8-10
FAO, The United Nations	15-30	–	3-7	–

Source: Dimić, 2005

The oil content of grape seeds may considerably vary. This actually depends on a variety, degree of grape ripeness followed by climatic and growing conditions (Kinsella, 1974; Rice, 1976; Dimić, 2005; Luque-Rodríguez *et al.*, 2005; Crews *et al.*, 2006; Matthäus, 2008; Choi and Lee, 2009; Mironeasa *et al.*, 2010; Pardo *et al.*, 2011). It ranges from 6 to 20%. The oil contents of the seeds of black and white varieties range from 16-19 and 15-18%, respectively. Furthermore, the oil content of the seeds of European varieties ranges from 12 to 18%, while domestic varieties contain about 13%. Grapeseed oil contains about 90 and 10% of the unsaturated and saturated fatty acids, respectively (Luque-Rodriguez *et al.*, 2005; Crews *et al.*, 2006; Bail *et al.*, 2008; Matthäus, 2008; Pardo *et al.*, 2009; Rubio *et al.*, 2009). Linoleic acid is the most common one (58-78%) followed by oleic acid (up to 22%, depending on the concrete variety) (Luque-Rodríguez *et al.*, 2005; Crews *et al.*, 2006; Matthäus, 2008; Bail *et al.*, 2008; Pardo *et al.*, 2009, 2011). Both fatty acids are essential since they cannot be formed in the body, but must be ingested through food. Linolenic acid, as well as palmitic and stearic acids, are present in much lower concentrations. The aim of this work was to briefly put some light both on the fatty acids and other naturally occurring compounds (natural products, secondary metabolites) reported thus far as the bioactive ingredients of the grapeseed oil-cold pressed.

Material and Methods

This mini review paper has been mostly prepared on the basis of the listed references below. More precisely, some additional experimental results of the Authors (yet unpublished) are also included.

Results and Discussion

A number of records from the available literature data (Miele *et al.*, 1993; Crews *et al.*, 2006; Pardo *et al.*, 2009; Rubio *et al.*, 2009), along with our own experimental findings, is briefly summarised (Table 2), when it comes to the fatty acids reported for the analysed grapeseed oil samples. The factors such as variety, growing area, grape ripeness, soil composition, oil production process, oil production conditions and the seed treatment prior to its processing are actually among the key ones known to affect the fatty acid composition of the grapeseed oil. There is a great similarity between this and sunflower oil. Compared to the pumpkin oil (34-65%), soybean oil (48-59%) or corn oil (34-65%), the grapeseed oil is found to be enriched with linoleic acid, an essential fatty acid that exhibits anti-inflammatory effects and may contribute to the prevention of thrombocytosis.

Table 2. Fatty acid contents of various grapeseed oil samples.

Fatty Acid	Miele *et al.*, 1993	Crews *et al.*, 2006	Pardo *et al.*, 2009	Rubio *et al.*, 2009	Experimental data of the Authors of this work (yet unpublished)
Caprylic	< 0.10	–	–	–	0.07
Lauric	< 0.10	–	–	–	0.05
Myristic	0.10	0-0.10	0.04–0.08	0.04-0.06	0.04
Palmitic	7.00	6.60-11.60	6.00–19.00	8.36-9.33	7.58
Palmitoleic	0.10	0.10-0.20	–	0.10-0.12	0.12
Margaric	–	0-0.10	–	0.07-0.09	0.06
Margaroleic	–	–	–	0.04-0.05	0.03
Stearic	4.20	3.50-5.40	4.42-5.87	3.26-4.36	4.62
Oleic	9.90	14.00-20.00	16.07-24.88	12.28-18.73	14.36
Linoleic	77.60	61.30-74.60	60.94-69.16	67.61-72.98	63.22
Linolenic	0.60	0.30-1.80	0.62-0.64	0.35-0.97	0.56
Gadoleic	0.20	0-0.04	–	0.13-0.16	0.11
Arachidic	0.20	0.10-1.70	0.17-0.21	0.18-0.33	0.17
Behenic	0.10	0.10-0.50	–	0.02-0.06	0.08
Erucic	–	0-0.10	–	0.02-0.13	0.06
Lignoceric	–	0-0.30	–	0.01-0.02	0.03

Source: Highlighted references

The same oil is rich in vitamin E or tocopherol, the substance capable to reduce the risk of arteriosclerosis leading to a lower cholesterol content (Dimić, 2005). As a consequence, it has potential to successfully target both cardiovascular diseases and problems with circulation (Pardo *et al.*, 2009). The grapeseed oil's tocopherols and tocotrienols are claimed as potent natural antioxidants likely to reduce oxidative stress at certain extent. Their contents in this type of oil range from 63 to 1298 mg/kg, as follows: α-tocopherol 7-229 mg/kg, β-tocopherol 4-133 mg/kg, γ-tocopherol 2-168 mg/kg, α-tocotrienol 10-352 mg/kg, β-tocotrienol 0-125 mg/kg and γ-tocotrienol 22-785 mg/kg (Pićurić-Jovanović and Milovanović, 2005; Beveridge *et al.*, 2005; Crews *et al.*, 2006; Bravi *et al.*, 2007; Matthäus, 2008). According to our own findings (yet unpublished), the same contents in the grapeseed oil may range from 86 to 558 mg/kg of a sample oil, as indicated herein: α-tocopherol 44-88 mg/kg, β-tocopherol 36-152 mg/kg, γ-tocopherol 24-78 mg/kg, β-tocotrienol 34-66 mg/kg and γ-tocotrienol 56-212 mg/kg. The sterol content of the grapeseed oil samples has also been the subject of intensive research work till date (Tiscornia and Bertini, 1973; Firestone, 1997; Beveridge *et al.*, 2005; Crews *et al.*, 2006; Matthaus, 2008; Pardo *et al.*, 2009; Rubio *et al.*, 2009). Both the grape variety and the drying temperature (the higher temperatures, the higher content) were correlated to this group of organic compounds.

The grapeseed's sterols are known to start decreasing at the onset of the ripening of the berry – veraison. After the veraison stage, it stabilises providing a constant level until the end of the full ripeness of the berry. The sterol content in this oil range from 285 to 1860 mg per 100 g

of the oil. In detail, cholesterol is in the range 0.2-0.4%, campesterol 9-14%, stigmasterol 2.2-17%, sitosterol 74.2-75.3% and avenasterol 1-3%. Taking into account our preliminary data, the sterol content of the grapeseed oils has ranged from 366 to 586 mg per 100 g of the oil. In one of the samples, cholesterol was 0.27%, campesterol 11.88%, stigmasterol 8.70%, while sitosterol and avenasterol were 73.76 and 2.14%, respectively.

Phenolic compounds (ranging from 11 to 116 μg/g) have been reported for this type of oil, too (Bail *et al.*, 2008; Pardo *et al.*, 2009). In comparison, our findings have pointed out the range 48-86 μg/g. Consequently, the antioxidant capacity of the various grapeseed oil samples has been screened. In difference to our findings (0.58-0.92 μg TE/g of a sample), the previously available literature data claim a broader range (0.10-1.16 μg TE/g of a sample). A high positive correlation has been clearly seen between the content of phenolic compounds (in the grapeseed oil) and its antioxidant capacity. In addition to this, the unrefined oils, along with the oils obtained from the black grape varieties, represent a rich source of phenolic compounds.

Finally, the pharmaceutical industry has launched this oil as the excellent carrier of many products, including face creams, lotions, lip and hair balms, etc. Also, the grapeseed oil is claimed to hydrate quite well both the skin and hair.

Table 3. The fatty acid contents of some vegetable oils.

Oil Sample	Linoleic Acid (%)	Linolenic Acid (%)	Oleic Acid (%)	Saturated Fatty Acids (%)
Linseed	15-24	40-60	14-25	7-16
Walnut	69	3	17	11
Hemp	53-68	16-24	6-12	8-10
Soybean	49	4	34	13
Sunflower	57	–	34	–
Tobacco	20-60	–	20-35	10-40
Corn	41	–	45	11
Beet	14-15	–	20-23	–
/ Grape Seed	69	–	16	15

Source: Rac, 1949

Conclusions

Taken all together, the grapeseed oil-cold pressed might be seen as the superior one compared to a number of other vegetable oils, both in terms of its quality and unique characteristics. High contents of linoleic acid and vitamin E do favour its regular use in the diet of the population facing with hypertension (high blood pressure) and/or the spectrum of heart disease problems.

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PLANT PROTECTION AND FOOD SAFETY

CONTAMINANTS IN VEGETABLE OILS

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Abstract

Vegetable oils are an extremely important ingredient in a proper diet, primarily because they are a significant source of energy, essential fatty acids, minor components and liposoluble vitamins. In addition to the above, edible oils also have certain physiological functions. Edible oil is a product used to prepare food and aromas, as well as food that does not require heat treatment. Edible vegetable oils are triacylglycerols, in smaller quantities containing other minor components that are natural ingredients of the oil. They are indispensable in the preparation of meals as well as in the production of numerous food products. It is important that the oils are of high quality and shelf life in order to fulfill their multiple roles. Crude oils can also contain various contaminants such as: pesticides, polycyclic aromatic hydrocarbons (PAHs), mycotoxins, mineral oils, etc. They can be easily accumulated in the oil, during vegetation, processing and storage of raw materials (oilcrops). All these chemical contaminants and their metabolites are: carcinogenic, teratogenic and immunotoxic, having a significant impact on human health. Therefore, more and more attention is paid on analytical methods for their determination. The risk of contamination and its type depends on the type of oil and its origin. Information on their presence in different oils is collected in three ways: by monitoring all phases of crude oil production, testing of crude oil and disseminating information on contaminants in oil production and science. These contaminants are removed at different stages of the refining process. Therefore, these components should not be present in refined edible oils.

Keywords: *Edible oils, Pesticides, Polycyclic aromatic hydrocarbons (PAHs), Mycotoxins, Rafination.*

Introduction

Oils and fats have been used since ancient times for food preparation, and they also have non-food applications such as lamp oil, lubricants, soap production and skin care. Oils and fats provide functionality in the preparation and use of food and contribute to improving the nutritional quality of prepared food. They serve as a heat transfer medium at elevated temperatures (eg frying), improve the taste of food (spreads and dressings), give texture and taste to a wide range of foods, they are a rich source of energy, essential fatty acids and essential minor components such as vitamins A and D. A balanced intake of oils and fats is important for human health. The production of vegetable oils and fats consists of: growing oilseeds, fruits, grains or nuts, oil extraction, refining and modifications to optimize oil properties and transport from growers to final user (van Duijn, 2014).
Until the Industrial Revolution in the 19th century, oilcrops, flaxseed, olives and walnuts were the main sources of vegetable oils. Today, the world market is dominated by palm and soybean oil, followed by rapeseed and sunflower oil. This led to a change in the extraction process and the purification/modification processes. Initially, the oil extraction process consisted of cleaning, crushing, heating and pressing. From 1900 onwards, solvent oil extraction was further applied from the cake to separate the oil remaining in the cake after pressing, or mechanical extraction was completely replaced by chemical (eg for soybean oil).

The process of oil purification was changed from simple decantation and filtration to a combination of neutralization with hydroxides (sodium), bleaching with active soil and deodorization at high temperature under vacuum in the presence of water steam. Using the refining process, the content of minor components is reduced in order to improve taste and appearance, while after the introduction of this process, the removal of solvent residues and nickel catalyzed hydrogenation is required. Later, the introduction of improved analytical techniques showed that the refining process reduces the content of many contaminants present in the crude (extracted) oil (eg pesticides, polycyclic aromatic hydrocarbons (PAHs), dioxins, mycotoxins, mineral oils, etc). The refining process can also produce by-products of side reactions, some of these products can affect health.

Pesticide residues

Plant protection products or pesticides can be used during the cultivation of plants, in the vegetation phase, where the plants are protected from various pests, adverse weather conditions, weeds or during storage of seeds, fruits, berries, as well as during transport.
The EU proposes limits on the amount of pesticides, in order to avoid undesirable outcomes. Also, the limits for pesticide residues in agricultural products have been harmonized. The maximum residue limit (MRL), ie pesticide residues that can be found in products after the application of pesticides according to good agricultural practice (GAP), is also given (Figure 1) (van Dujin, 2016).

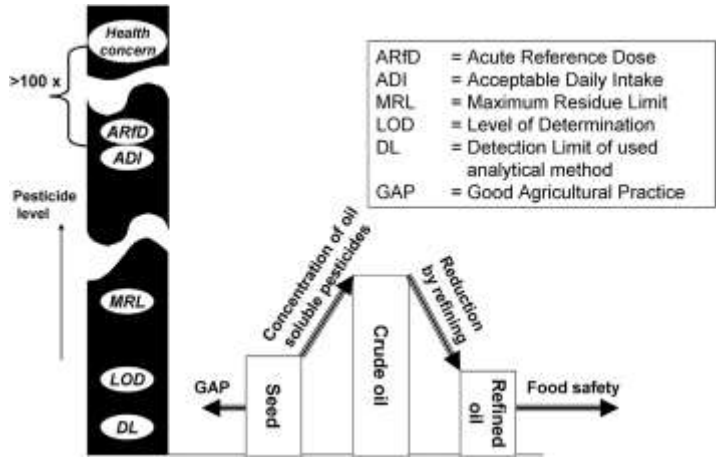


Figure 1. Qualitative changes in pesticide levels by extraction and subsequent refining of oil (vertical column shows the relationship of different health limits, legal limits and detection limits) (van Dujin, 2014)

Despite these suggestions, pesticide residues may appear in vegetable oils. Depending on the physical and chemical properties, the pesticide residues present in the oil will be concentrated depending on the way the oil is extracted. It will be concentrated in crude oil if the pesticides are soluble in oil or hexane. The maximum concentration factor of oil-soluble or hexane-soluble pesticides, X (max), can be calculated based on Eq. 1 where C *oil* is the oil content of the seed.

$$X\ (max) = \frac{100}{C oil} \quad (1)$$

During the extraction of palm oil, pesticides, if they are soluble in water, are concentrated in the sludge. The concentration of pesticides in the oil and in the obtained cake (meal) is equal to the level of pesticides in the seed.

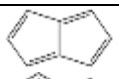
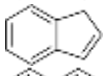
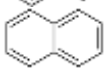
The pesticides used or most commonly found in oil are mainly organophosphorus insecticides or organochlorine pesticides. All of them are removed to below the limits of detection in the processes of chemical and physical refining (van Duijn, 2016). They are used to protect seeds during transport and storage (post-harvest treatment). Pesticides found in soybean and sunflower oils are: fenitrothion, melation, perimiphos-methyl, dichlorvos, endosulfan, etc. The highest level of pesticides and the frequency of their occurrence was found in sunflower oils, while significantly lower amounts of rapeseed and soybean oil were present. Palm oil is processed in a few days, preferably within 24 hours after harvest to preserve quality. Pesticides are not found in these oils, nor in coconut oil, because they are very often not used in the production of these raw materials (van Duijn, 2014).

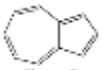
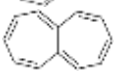
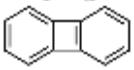
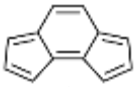
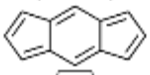
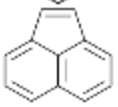
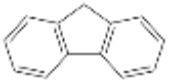
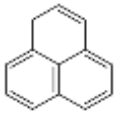
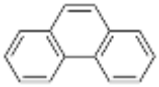
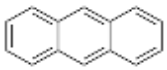
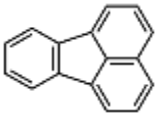
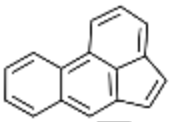
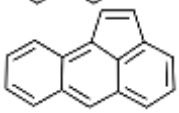
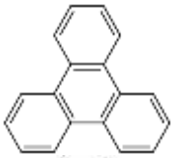
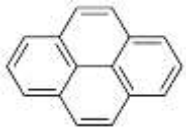
Polycyclic aromatic hydrocarbons (PAHs)

Crude oils are a mixture of hydrocarbon fractions, with different solubilities. Among them, PAHs rank among relatively more soluble compounds compared to alkanes, which contain the same number of carbon atoms. Their solubility is a very important characteristic, since they are classified as the most harmful contaminants of crude oils. The chemical composition of crude oil varies greatly depending on the raw materials obtained from, which is the reason of different effect on ecosystem organisms. Differences occur due to different toxicity and concentration of certain compounds. PAHs are a large group of organic compounds consisting of two or more fused aromatic rings. They are primarily formed by incomplete combustion of organic matter. For many of them, there is clear evidence of mutagenicity, as well as genotoxic and carcinogenic effects, which has been studied in animals. Commonly used markers for PAH molecules in crude oil are benzo (a) pyrene (BaP) and heavy PAHs with four aromatic rings (PAH4). High levels of BaP were analyzed in coconut and sunflower oil. The EU provides regulations to limit the PAH content for products that involve drying or frying in the production process. The maximum BaP content for oils and fats used for food preparation is 2 μg/kg, and for PAH4 10 μg/kg of product. For coconut oil, the PAH4 content is slightly higher and amounts to 20 μg/kg. During deodorization, light PAH molecules are removed between 50% and 80% at a temperature of 180°C. Heavy PAHs are removed with activated carbon, as well as concentrated BaP. Much of the light PAH will be concentrated in the used bleaching earth and the other part in the distillate (Baek et al., 2004; Ramachandran et al., 2006; van Duijn, 2016).

Table 1 shows some of the PAHs that can be found in crude oil. However, in addition to oil, they can also be found in the environment, due to the use of fuel (diesel), which produces combustion. They are also released due to the burning of coal, wood, and tobacco. The US Environmental Protection Agency (USPEA) has identified 16 PAH compounds as priority pollutants. Some of them are: naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, pyrene, etc. PAH molecules are also found in food. Studies have shown that PAHs in food are most abounded in cereals, oils and fats, and smaller amounts can be found in vegetables. BaP is the first toxic aromatic hydrocarbon to be discovered and is found in tobacco smoke (Kumar et al., 2011).

Table 1. Polycyclic aromatic hydrocarbons present in crude oil (Kumar et al., 2011)

No.	Radial depiction	Name	Structure	Molecular formula
1	Pen	Pentalene		C_8H_6
2	Ind	Indene		C_9H_8
3	Nap	Naphthalene		$C_{10}H_8$

4	Azu	Azulene		$C_{10}H_8$
5	Hep	Heptalene		$C_{12}H_{10}$
6	Bip	Biphenylene		$C_{12}H_8$
7	aIn	as-Indacene		$C_{12}H_8$
8	sIn	s-Indacene		$C_{12}H_8$
9	Can	Acenaphthylene		$C_{12}H_8$
10	Flu	Fluorene		$C_{13}H_{10}$
11	Phe	Phenalene		$C_{13}H_{10}$
12	Phr	Phenanthrene		$C_{14}H_{10}$
13	Ant	Anthracene		$C_{14}H_{10}$
14	Flt	Fluoranthene		$C_{16}H_{10}$
15	Acp	Acephenanthrylene		$C_{16}H_{10}$
16	Aca	Aceanthrylene		$C_{16}H_{10}$
17	Tpl	Triphenylene		$C_{18}H_{12}$
18	Pyr	Pyrene		$C_{16}H_{10}$

Dioxins, furans, dioxins as PCBs and heavy metals

Dioxins include the following compounds: polychlorinated dibenzodioxins (PCDDs) (dioxins); polychlorinated dibenzofurans (PCDFs) (furans); polychlorinated biphenyls (PCBs) and dioxin (2,3,7,8-TCDD), as the most toxic.

The chemical name for dioxin is: 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD). About 419 dioxin-type compounds have been identified, but only 30 have significant toxicity.

Dioxins can be formed in nature, during forest fires, due to volcanic eruptions, incomplete combustion of organic matter, as well as from industrial chemical processes (production of pesticides and chemicals, production of metals), as by-products. They are highly lipophilic, which gives them the ability to be absorbed into adipose tissue and have a biological half-life

in the human body from 7 to 11 years (Bell et al., 2005). Dioxins can be found in the air (derived from the combustion of medical, municipal, hazardous and household waste), in soil, water, sediments, and in foods such as meat, dairy products, fish and shellfish.

The World Health Organization (WHO) has established a permitted monthly intake (Provisional Tolerable Monthly Intake (PTMI)) for dioxins, furans and PCBs of 70 pg/kg body weight. This value represents the permitted intake during life which does not pose a risk to human health.

Exposure to dioxins, furans and other similar compounds mainly comes through the food chain, and PTMI (permitted monthly intake) is a cumulative exposure to dioxins and furans from all sources, including food and water.

The levels of these contaminants in the extracted vegetable oils are significantly below the permitted limits. Much higher levels, compared to these oils, are found in fish oil with their allowable content in fish oil being slightly higher. Several studies have been performed to reduce their content in refined fish oil. The conclusions of this research are (van Dujin, 2016): dosing of activated carbon during bleaching removes dioxins and furans and reduces the content of PCBs and reduction of dioxins, furans and PCBs during deodorization depends on the temperature. The reduction is increased from 10 to 25% at a temperature of 180°C, and a temperature of 220°C is required for almost complete removal.

The EU has issued regulations for the maximum content of these contaminants in oil. The limit for dioxins in vegetable oils and fats is 0.75 pg/g and 1.5 pg/g for a mixture of dioxins and PCBs. The limits for fish oil are slightly higher and are 2 pg/g for dioxins and 10 pg/g for a mixture of dioxins and PCBs (van Dujin, 2016).

Dioxins are extremely toxic and can cause problems in reproduction, can damage the immune system, interfere with the work of hormones and can cause cancer. Dioxins are increasingly present, so people are exposed to their effects, but in concentrations that do not affect health.

The heavy metals most commonly found in crude oil are copper (Cu), mercury (Hg), manganese (Mn), lead (Pb), arsenic (As), zinc (Zn), and cadmium (Cd). Some of them are necessary for the normal functioning of metabolism, such as: Zn, Cu, Fe. However, many of them are toxic and in small quantities, carcinogenic such as. Pb, As, Hg, Cd, Pt (Lacoste et al., 1999). These metals have shown a tendency to accumulate in the human body, causing irreversible damage. Another harmful effect is on the environment. The metals found in oils are mainly transported through soil, air during vegetation or storage, as a result of environmental pollution.

Hydrocarbons of mineral origin

Hydrocarbons of mineral origin usually consist of linear or branched chains of paraffinic, non-paraffinic or aromatic hydrocarbons. The number of carbon atoms ranges from 5 to 55. Usually, the physical properties of mineral oils depend on their composition in terms of the number of C atoms and their distribution. Therefore, the physical properties are very different, so the boiling points range from 300 to 600°C, while the relative density ranges from 0.820 for light paraffinic, base oils, to just over 1 for highly aromatic base oils. All of them are mostly insoluble in water and alcohol, and soluble in hexane, chloroform, ether, petroleum ether, etc. They are formed from crude mineral oils in various stages of refining, such as distillation, extraction or crystallization followed by purification. They can be divided into several groups depending on the number of C atoms and/or viscosity. They are classified according to the number of C atoms: C6 - hexane, used as a solvent in the extraction of vegetable oils; C6-C10 - gasoline; C10-C24 - middle fraction, consisting of kerosene, diesel and light fuel oil; C20-C55 - medium and very viscous oils such as fatty oils, hydraulic oils, etc. Oils and fats used for the preparation or in the process of food production must be without hydrocarbons of mineral origin (van Dujin, 2014).

Mineral oils are used through motor oils, industrial and automotive gear oils, fluid transmission, hydraulic fluids, carrier and machine oils. They are also used as textile oils, metalworking oils, as anti-corrosion oils. They can also be used in medicine (paraffin oils) (Aluyor and Ori-jesu, 2009).

Based on the research, the FAO/WHO has accepted a certain daily intake of mineral oils with a high viscosity of max 20 mg/kg body weight, and for medium and low viscosity mineral oils 10 mg/kg body weight. Mineral oil products can be found in crude edible oil due to contamination during production (lubricants and hydraulic oils), as residues from previous cargo during transport, storage or as a false addition of some other oils. However, their presence may also be the result of the permitted use of various substances such as hexane for extraction, as pesticide protection agents or protection in seed storages (van Duijn, 2014).

Mycotoxins

Agricultural products used as food or raw materials for food products are always exposed to the danger of contamination by fungi during cultivation, harvest, transport and storage when the environment, temperature and humidity are suitable for the growth of certain species. About 200 different species of filamentous fungi, such as *Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria* can produce mycotoxins. Mycotoxins are natural, secondary metabolites of fungi that are synthesized from a large number of biochemically simple intermediates of primary metabolism (acetate, malonate, serine, tryptophan, alanine) due to the activity of various enzymes. So far, several hundred different mycotoxins have been discovered that show great structural diversity, resulting in different physical and chemical properties. Some of the most common mycotoxins are: aflatoxin and ochratoxin (produced by *Aspergillus* spp.), fumonisins, trichothecenes, zearelenone (produced by *Fusarium* spp.), patulin (*Penicillium* sp.). Mycotoxins are potent toxins and have a wide range of effects on animals and humans. They are: cytotoxic, neurotoxic, nephrotoxic, carcinogenic, mutagenic, immunosuppressive, teratogenic. Although mycotoxicosis caused by direct consumption of contaminated food poses the greatest risk to humans, the possibility of their ingestion through milk, meat or eggs must also be considered (Krska et al., 2009).

According to the degree of toxicity, mycotoxins are divided into three groups (Kocić-Tanackov and Dimić, 2013):

- extremely toxic: cyclochlorothin and rubatoxin B (lethal doses less than 1 mg/kg body weight);
- very toxic: aflatoxin, trichothecenes and citreoviridine (lethal doses of 1-10 mg/kg body weight) and
- other toxic metabolites (lethal doses greater than 10 mg/kg body weight).

In recent years, vegetable oils have been used more and more, and they have gained more popularity than animal fats. Also, the use of cold-pressed and virgin oils, which have not undergone the refining process, is increasing. Due to the danger of contamination, and their bad impact on the body, the maximum doses that can be found in oils without negative consequences on the body are given. Of course, the permitted values are different for different mycotoxins.

Influence of refining process on oil contaminants

The technological process of refining crude oils is a complex process of removing certain components in order to obtain the highest quality of edible oil. This process consists of a series of technological procedures where certain components are removed. In addition to removal, there are some components of vegetable oils that should be preserved, due to their positive effect on the human body, but also the oil itself. During the refining process, as with other technological processes, certain by-products are created. Today, more and more efforts

are being made to process improvement from the economic aspect and from the aspect of environmental protection. In addition to secondary products, various contaminants can be found in the oil, which were previously described. They need to be detected and then removed from the oil. They are removed through various stages during oil refining.

Pesticides used to protect plants in vegetation may be present in oils. It is very important to use the prescribed doses of pesticides, so that large amounts of pesticides are not found in the oils themselves. The amounts of pesticides in oils can be determined by HPLC (liquid chromatography under high pressure) method or mass spectrometry. The concentration of pesticides decreases in all phases of refining. The effect of each phase depends on the physicochemical characteristics of the pesticide. Water-soluble pesticides dissolve in alkaline solution during neutralization and are removed with soaps. Some pesticides are absorbed by acid-base interactions with the bleaching soil during the oil bleaching phase. Volatile pesticides are removed during deodorization, as are all organophosphorus pesticides. Removal is performed at temperatures around 220-270°C (van Dujin, 2014).

PAHs in oils are detected by the same methods as pesticides, using HPLC methods and gas chromatographic analysis. They are removed by dosing activated carbon in the process of oil bleaching, as well as in the process of deodorization at high temperatures. Volatility depends on the number of aromatic rings in the PAH molecule. During deodorization, about 50% of the PAH compound is removed at 180°C and about 90% of the PAH compound at 240°C.

Dioxins, furans and heavy metals are also detected by these methods, as well as previous contaminants. Dioxins are removed from the oil in the stages of blanching (sterilization, eg in palm fruits) and deodorization (at a temperature of about 180°C). Heavy metals are rarely present within the limits of detection in crude oils. Therefore, the refining process cannot be counted as a process for heavy metals removal, except for Fe and Cu, which are effectively removed by neutralizing and bleaching the oil. The EU has regulated only the level of Pb in oil and it amounts to a maximum of 0.1 mg/kg of body weight (van Dujin, 2014).

Hydrocarbons of mineral origin such as hexane are removed by the neutralization and bleaching process, while the residue is removed during deodorization to levels below the detection limits (0.1 mg/kg). The upper limit for the residual hexane in the oil is 1 mg/kg. Volatile compounds are removed during deodorization.

Mycotoxins are detected in oils by chromatographic methods. Depending on the type of mycotoxin, they are removed in different stages of refining, but all are removed below the level of detection, ie 1 μg/kg. Aflatoxins are removed in the phases of neutralization and bleaching. In the bleaching process, the bleaching earth is responsible for removing aflatoxins. Aflatoxins are removed in insignificant quantities in the deodorization phase, precisely because they can withstand high temperatures. Also, for the removal of some mycotoxins, chemical refining is better (removal of zearalenone is more efficient in chemical refining, about 80-98%) compared to physical refining (about 70-80%) (van Dujin, 2014).

Table 2 shows the effect of physical and chemical refining on the various contaminants in the oils. It is noticeable that chemical refining is somewhat more successful when it comes to removing contaminants from oil, however the level of contaminants will be reduced within the allowed limits by applying both refining procedures.

Table 2. Content of contaminants and removal phases (van Duijn, 2014)
* - chemical refining

Contaminants	Hydrocarbons of mineral origin (mg/kg)	PAH (BaP) (µg/kg)	Pesticides (mg/kg)	Aflatoxin B1 (µg/kg)	Zearalenone (µg/kg)
Max allowed quantities in oils	0,1-1	2		2	400
Pre-refining					
Neutralization			**	*	*
Bleaching	*	*	**	***	***
Deodorization	*	*	*		

** - chemical and physical refining
*** - physical refining

Table 3 provides an overview of the maximum allowed concentrations of individual contaminants in vegetable oils, oilseeds and crude oils, according to the regulations of the Republic of Serbia (Rulebook, 2013; Rulebook, 2018; Rulebook, 2019).

Table 3. Maximum allowed concentrations of individual contaminants in vegetable oils according to the regulations of the Republic of Serbia

Mycotoxins/Aflatoxins	Maximum allowed concentration (µg / kg)		
	B_1	Sum of B1, B2, G1 and G2	M_1
Peanuts and other oilseeds that are subject of sorting or otherwise physically (mechanically) processed before use for human consumption or for use as a food ingredient, except: - Peanuts and other oilseeds intended for crushing and production of refined vegetable oil	8.0	15.0	-
Peanuts and other oilseeds and their processed products intended for direct human consumption or use as food ingredients, except: - crude vegetable oils intended for processing and - refined vegetable oils	2.0	4.0	-
Metals and non - metals/Lead	Maximum allowed concentration (mg/kg wet weight)		
Fats and oils, including milk fat	0.10		
Glycidyl esters of fatty acids expressed as glycidol	Maximum allowed concentration (µg/kg)		
Vegetable oils and fats placed on the market for the final user or for use as a food ingredient	1.000		

Dioxins and polychlorinated biphenyls (PCBs)	Maximum allowed concentration		
	Sum of dioxins	The sum of dioxins and dioxin-like PCBs	Sum of PCB 28, PCB 52, PCB 101, PCB 138, PCB 153 and PCB 180
Vegetable oils and fats	0.75 pg/g masti	1.25 pg/g masti	40 ng/g masti
Polycyclic aromatic hydrocarbons/ Benzo(a)pyrene, benzo(a)anthracene, benzo (b) fluoranthene and chrysene	Maximum allowed concentration (μg/kg)		
	Benzo(a) pyrene	Sum of benzo(a)pyrene, benzo(a) anthracene, benzo(b)fluoranthene and chrysene	
Oils and fats (other than cocoa butter and coconut oil) intended for immediate consumption or for use as a food ingredient	2,0	10,0	
Cocoa beans and cocoa bean products	5.0 μg/kg fat	30.0 μg/kg fat	
Coconut oil intended for immediate consumption or for use as a food ingredient	2.0	20.0	
Specific plant toxins/Erucic acid	Maximum allowed concentration (g/kg)		
Vegetable oils and fats	50		
Food containing added vegetable oils and fats	50		
Residues of plant protection products/Pesticides	Maximum allowed concentration of residue (mg/kg) indicated for about 400 active substances in most cases 0.01-0.05		
Mineral oils/Solvent residue	Maximum allowed concentration (mg/kg) nor in traces (for all types of edible oils)		

Conclusions

The use of fats and oils in the human diet has become increasingly popular and important in recent years. The reasons are multiple: high energy value and intake of essential fatty acids and liposoluble vitamins. In addition to good components, some undesirable components, contaminants, can be found in oils. The most common contaminants are mycotoxins, polycyclic aromatic compounds, mineral oils, heavy metals, dioxins, pesticides. All these components have a negative impact on human health and therefore must be removed from the oil, ie reduced to quantities that are not dangerous to human health. Concentrations of these components are usually determined by the World Health Organization (WHO) and similar organizations that deal with this issue, precisely because of their harmful effects on both human health and the environment. All of these chemical contaminants and their metabolites are carcinogenic, teratogenic, immunotoxic, neurotoxic, or mutagenic.

The technological process of refining crude oils is a complex process of removing certain components in order to obtain the highest quality edible oil, but also health-safe oil. This process consists of a series of technological procedures where certain components are

removed. Different contaminants are removed in different stages of refining process, depending on their physical and chemical characteristics. Research has shown that chemical refining is somewhat more successful than physical refining. The stages of refining where most contaminants are removed are neutralization, bleaching and deodorization. During neutralization, some pesticides, hydrocarbons of mineral origin, mycotoxins, heavy metals are removed. During the bleaching phase, due to the use of bleaching earth, pesticides, PAH molecules and mycotoxins are removed. Deodorization at elevated temperatures removes pesticides, hydrocarbons of mineral origin, polycyclic aromatic hydrocarbons, furans. Since many contaminants are removed in several stages of refining, it is certain that they will be removed from the oil.

In addition to removal, some components of vegetable oils need to be preserved, due to their positive effect on human health, but also on the quality of the oil. Therefore, it is very important to conduct all technological procedures during the refining process. Also, when applying pesticides or other auxiliary components during the vegetation, it is important to use them in the allowed quantities, in order to avoid the occurrence of increased amounts in the raw material, and thus in the oil as the final product.

Acknowledgments

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PHYSICOCHEMICAL PROPERTIES OF HONEY FROM THE ENTITY OF REPUBLIC OF SRPSKA (BOSNIA AND HERZEGOVINA)

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Abstract

The present study aimed to evaluate physicochemical characteristics and quality of honey from the entity of Republic of Srpska, Bosnia and Herzegovina. For this purpose, 19 samples of *Apis mellifera* honey of different floral types were obtained from different locations in entity of Republic of Srpska, collected in the period 2016-2019. Reducing sugars, saccharose content, moisture, free acidity, ash, electrical conductivity, hydroxymethylfurfural (HMF), pH and diastase activity (DN) were analyzed using recommended methods to satisfy regulatory requirements.The efficiency of the qualitative tests (Fiehe's test, Lugol's reaction, Lund's reaction) was tested.The quality of the honey types varied, based on botanical origins, and presumably, handling and storage conditions. Among the overall determined parameters diastase activity and free acidity in one honey was not acceptable according to national and international regulations. Also, all qualitative tests were negative, indicating that all samples were of natural origin, without adulteration. The regression analysis clearly indicated that acidity in acacia honeys is highly correlated with electrical conductivity, diastase activity and moisture, but negatively correlated with HMF content. Also, the positive correlation of electrical conductivity, moisture and diastase activity, and negative with HMF content was observed.

Keywords: *Honey, physicochemical properties, quality.*

Introduction

Honey has been defined as a natural sweet mixture produced by honeybees from the nectar of flowers or from living parts of plants (Montenegro and Mejías, 2013) and has various medicinal properties such as antibacterial, antihypertensive, hepatoprotective, hypoglycemic and antioxidative activity (Erejuwa *et al.*, 2012). Honey represents complex of various compounds of plant and honeybee origin such as sugars, proteins, enzymes, amino acids, vitamins, hormones, flavonoides, inorganic acids and trace elements. Honey consists mainly of carbohydrates (at least 60% in mass ratio), particularly reducing sugars such as fructose and glucose as fast energy source upon consumption (Chua and Adnan, 2014). The water content (moisture) is especially important, and it ranges from 14 to 18% in natural fresh honey. As long as the water content remains below 18%, virtually no microorganisms can successfully multiply in honey (Rehman *et al.*, 2008). The properties and composition of honey can vary widely depending on the region, season, bee variety, plant source of nectar and storage time in the honeycomb, mode of harvesting and post-harvest storage etc. (Kukurová *et al.*, 2008; Sereia *et al.*, 2017). Depending on the biological source from which it is obtained, honey can be floral honey and honeydew honey (forest honey). The biological source of floral honey refers mainly to nectar produced by flowers of various plants (Jovanović, 2015). Pure acacia honey (*Robinia pseudoacacia*), without any additions, is light-coloured, glassy and transparent, almost colourless and with a weak intensity of odour. Its taste is reminiscent of acacia sap, and it is very mild and pleasant. Mature acacia honey is thick, sticky at low temperatures and stretchy like dough (Jovanović, 2015). Chestnut honey

(*Castanea sativa L.*) has a characteristic dark reddish colour and intensive taste which is often slightly bitter. This type of honey is very slow to crystallise (Oreški, 2014). Forest honey (honeydew honey) is made of sweet substances secreted by some insects as by-products, feeding on phloem sap of the plant on which they land (Jovanović, 2015). According to microscopic analyses, this type of honey is characterised by low content of pollen and elements of honeydew (Lazarević, 2016). Honey quality must be analytically controlled with the aim of guaranteeing its declared specifications and it is mainly determined by analysing the physicochemical and microbiological properties (Ćirić *et al.*, 2018). From the aspect of physicochemical indicators, honey quality in Bosnia and Herzegovina is defined by the Ordinance on honey and other bee products (Official Gazette of BiH, 37/2009 and 25/11). The aim of this work is to evaluate the physicochemical characteristics and quality of honey from Republic of Srpska, Bosnia and Herzegovina. For this purpose, samples of *Apis mellifera* honey of different floral types are obtained from different locations in entity of Republic of Srpska, collected in 2016-2019.

Material and Methods

A total of 19 different samples of honey produced in the territory of the entity of Republic of Srpska, Bosnia and Herzegovina, were used for the analysis: 5 samples of floral honey, 7 samples of acacia honey, 5 samples of chestnut honey and two samples of forest honey. The honey samples were produced in the period between 2016 and 2019. All the samples were provided in glass packaging and kept at the room temperature. Physicochemical analysis: The characteristics and satisfactory quality of all Samples were assured through an analysis of the following parameters: water content, diastase activity, HMF content, acidity, reducing sugars, sucrose, ash content, electrical conductivity as described by Ordinance on methods for control of honey and other bee products (Official Gazette of BiH no 37/2009), and Fiehe's test, Lugol's and Lund's reactions (Almeida-Muradian *et al.*, 2013). The pH was measured with a pH meter (HI-2211, Hanna Instruments, USA). All the chemicals and reagents used were of analytical grade. Table 1 shows the minimum honey quality requirements, in accordance with the regulations in force in Bosnia and Herzegovina.

Table 1. Minimum honey quality requirements

Parameter	Criteria
Sucrose content	In general: Not more than 5 % Forest honey - Not more than 10 %
Reducing sugars	Blossom honey ≥ 60 % Honeydew (forest) honey, blends of honeydew (forest) honey with blossom honey ≥ 45%
Moisture	In general: Not more than 20 %
Electrical conductivity	In general: Not more than 0,8 mS/cm Forest honey, Chestnut honey: not less than 0,8 mS/c,
HMF	In general: Not more than 40 mg/kg
Diastase activity	In general: Not less than 8
Ash content	In general: Not more than 0,6 g/100g, Forest honey - not more than 1,2 g/100g
Free acidity	In general: Not more than 50 meq/kg

*Source: Ordinance on honey and other bee products (Official Gazette of BiH, 37/2009 and 25/11)

All tests were performed in triplicate and the results were expressed as means ± standard deviation. Pearson's correlation (r) was used to determine relationships among different physico-chemical parameters.

Results and Discussion

Table 2 shows the content of total sugars, reducing sugars and sucrose in the tested honey samples. The average total sugar content (%) ranged from 70.25 ± 0.48% (forest honey) to 73.37 ± 5.24% (acacia honey), and the average content of reducing sugars ranged from 68.00 ± 0.66% (forest honey) to 71.70 ± 1.65% (chestnut honey). The sucrose content ranged from 1.61 ± 1.44% (chestnut honey) to 4.34 ± 2.60% (floral honey). The obtained values of these parameters are in accordance with the relevant legislation (Official Gazette of BiH, 37/2009 and 25/11).

Table 2. Content of total sugars, reducing sugars and sucrose (content ± standard deviation)

Botanical origin	Total sugars (%)	Reducing sugars (%)	Sucrose (%)
Floral (n=5)	72.43 ± 7.77	68.09 ± 6.18	4.34 ± 2.60
Acacia (n=7)	73.37 ± 5.24	71.06 ± 5.93	2.31 ± 1.49
Chestnut (n=5)	73.31 ± 1.97	71.70 ± 1.65	1.61 ± 1.44
Forest (n=2)	70.25 ± 0.48	68.00 ± 0.66	2.26 ± 0.18

Source: Author s' elaboration based on the obtained results

Carbohydrates are the major constituents of honey, corresponding to 95–99% of the dry matter (Olaitan *et al.*, 2007). The reducing sugars fructose and glucose make between 88% and 95% of the total carbohydrates, and they have the greatest impact on the physical properties of honey, particularly on thickness, viscosity, stickiness, tendency to crystallise, hygroscopicity and microbiological activity (Čalopek *et al.*, 2016; Vahčić and Matković, 2009). Gomes *et al.* (2010) stated that higher content of sucrose in honey might be due to an early harvest of honey, i.e. before sucrose has been converted into fructose and glucose.

Table 3. Water content and electrical conductivity (content ± standard deviation)

Botanical origin	Water content (%)	Electrical conductivity (mS/cm)
Floral (n=5)	17.10 ± 0.64	0.451 ± 0.106
Acacia (n=7)	16.68 ± 0.61	0.256 ± 0.104
Chestnut (n=5)	17.32 ± 0.86	1.111 ± 0.127
Forest (n=2)	16.68 ± 1.38	1.682 ± 0.844

Source: Author s' elaboration based on the obtained results

Table 3 shows the results of the analysis of the water content and electrical conductivity in the tested honey samples. The water content in the samples was significantly below the values allowed by the relevant Ordinance (Official Gazette of BiH, 37/2009 and 25/11), which provides that it may not exceed 20%. Salazar *et al.* (2017) stated that the analysis of water content in honey is very important due to the effect of this variable on the taste, viscosity and fluidity of this product. The moisture content has a direct impact on the activity of microorganisms, due to possible occurrence of fermentation (Salazar *et al.*, 2017, Gomes *et al.*, 2010), and it also affects the shelf life of honey (Salazar *et al.*, 2017). Gomes *et al.* (2010) stated that high water content can accelerate crystallisation and increase the water activity value, thus enabling the growth of yeasts. The moisture contents of tested samples were similar to previously reported moisture levels in some European honeys (Oddo and Piro, 2004; Lazarevic *et al.*, 2012; Matovic *et al.*, 2018; Ćirić *et al.*, 2018).

The electrical conductivity of the examined honeys is shown in Table 3. The lowest mean electrical conductivity was obtained for acacia honey (0.256±0.104 mS/cm) and the highest mean electrical conductivity was measured in forest honey (1,682 ± 0,844 mS/ cm). Based on the relevant Ordinance (Official Gazette of BiH, 37/2009 and 25/11), electrical conductivity for chestnut honey and forest honey must be at least 0.8 mS/cm, while this value is the maximum allowed for other types of honey. The obtained results show that all the values are in accordance with the prescribed values. Electrical conductivity reflects the mineral content in honey, and a high level of ash content is related to high electrical conductivity (Ngoi, 2016). Electrical conductivity is a good criterion for the botanical origin of honey (Adenekan *et al.*, 2010; Buba *et al.*, 2013; Kirs *et al.*, 2011) and according to Acquarone *et al.* (2007) and Gomes *et al.* (2010) electrical conductivity is directly related to the concentration of mineral salts, organic acids and proteins. Electrical conductivity of honeydew honey is higher in comparison with other types of honey, due to its origin (Escriche *et al.*, 2007). This study confirmed that as well, showing that chestnut honey and forest honey have higher acidity values and higher ash content, and the values of electrical conductivity are therefore higher in comparison with acacia honey and floral honey.

Table 4. HMF content and diastase activity of honey (content ± standard deviation)

Botanical origin	HMF (mg/kg)	DN (Schade units)
Floral (n=5)	7.26 ± 2.21	23.85 ± 11.44
Acacia (n=7)	8.99 ± 5.55	18.69 ± 8.72
Chestnut (n=5)	2.88 ± 2.25	31.17 ± 5.57
Forest (n=2)	1.78 ± 2.52	52.81 ± 11.39

Source: Authors' elaboration based on the obtained results

Table 4 shows the results of the measurement of the HMF content and diastase activity (DN). The HMF content in the tested samples ranged from 1.78 ± 2.52 mg/kg (forest honey) to 8.99 ± 5.55 mg/kg (acacia honey). The maximum value allowed by the relevant Ordinance (Official Gazette of BiH, 37/2009 and 25/11) is 40 mg/kg, which means that the HMF content in all the tested samples is in accordance with the relevant Ordinance. Buba *et al.* (2013) state that HMF is a product of fructose decomposition, and only traces of it can be found in fresh honey. However, its concentration increases with storage and prolonged heating of honey. Apart from that, other factors can also cause an increase in HMF content, such as pH, storage temperature, moisture, acidity, metals, amino acids (Buba *et al.*, 2013, Gomes *et al.*, 2010; Almeida-Muradian *et al.*, 2013). Janzowski *et al.* (2000) stated that high concentration of HMF has genotoxic effects and mutagenic potential on humans.

The diastase activity (DN) in the tested samples ranged from 18.69 ± 8.72 (chestnut honey) to 52.81 ± 11.39 (forest honey). According to the relevant Ordinance (Official Gazette of BiH, 37/2009 and 25/11), the DN value should be at least 8, which means that the values of all the samples are in accordance with the relevant Ordinance. Diastase is a natural enzyme found in honey. Like the HMF value, the diastase activity can be used as an ageing indicator (Gomes *et al.*, 2009), i.e. as a parameter for assessing the freshness of honey and/or its overheating (Kirs *et al.*, 2011, Buba *et al.*, 2013, Almeida-Muradian *et al.*, 2013). The diastase activity in honey varies widely depending on the botanical origin of honey, and HMF is therefore regarded as a better quality criterion in terms of honey freshness (Buba *et al.*, 2013).

Table 5. Ash content, acidity and the pH value (content ± standard deviation)

Botanical origin	ash (g/100 g)	acidity (mmol/kg)	pH
Floral (n=5)	0.15 ± 0.05	20.60 ± 5.39	4.00 ± 0.23
Acacia (n=7)	0.06 ± 0.06	14.90 ± 7.94	3.91 ± 0.07
Chestnut (n=5)	0.44 ± 0.03	26.00 ± 4.54	4.23 ± 0.09
Forest (n=2)	0.71 ± 0.48	68.09 ± 26.75	4.40 ± 0.20

Source: Author s' elaboration based on the obtained results

Table 5 shows the results of the analysis of the ash content, acidity and the pH value. The ash content for the analysed samples ranged from 0.06 ± 0.06 g/100 g (acacia honey) to 0.71 ± 0.48 g/100 g (forest honey). The maximum ash content allowed by the relevant Ordinance (Official Gazette of BiH, 37/2009 and 25/11) is 0.6 g/100 g, except for honeydew honey − 1.2 g/100 g. The obtained results show that the ash content in all the samples is in accordance with the relevant Ordinance. Salazar *et al.* (2017) stated that ash shows the mineral content in food, and as regards honey, the differences may be related to the floral source, the environment and the production and processing conditions. Almeida-Muradian *et al.* (2013) stated that the variability of ash content is related to the botanical and geographical origin of honey. They also state that floral honeys have lower ash content in comparison to honeydew honey, as shown by the obtained results.

The acid content ranged from 14.90 ± 7.94 mmol/kg (acacia honey) to 68.09 ± 26.75 mmol/kg (forest honey). The maximum value allowed by the relevant Ordinance (Official Gazette of BiH, 37/2009 and 25/11) is 50 mmol/kg, which indicates that forest honey is not in accordance with the prescribed quality. The acid content in honey is relatively low, but it is an important factor of the taste of honey. Most of acids in honey come from bees. The acidity of honey can be the result of the presence of gluconic, succinic, malic, acetic, citric, formic, lactic, folic and butyric acid (Salazar *et al.*, 2017). The main acid in honey is gluconic acid, which arises from the oxidation of glucose by the *glucose oxidase* enzyme (Buba *et al.*, 2013), and a high value of acidity can be an indicator of fermentation of sugar into organic acids (Gomes *et al.*, 2010; Almeida-Muradian *et al.*, 2013).

The relevant Ordinance (Official Gazette of BiH, 37/2009 and 25/11) does not foresee any measurement of the pH value of honey, which means that there are no limit values allowed for this parameter. The measured pH values ranged from 3.91 ± 0.07 (acacia honey) to 4.40 ± 0.20 (forest honey), and they are mainly in accordance with the values that can be found in the available literature. Kukurová *et al.* (2008) stated that the pH value for floral honey from Slovakia was 4.30, and the pH value for floral honey from Nigeria measured by Buba *et al.* (2013) ranged from 3.2 to 4.6. The pH value for chestnut honey measured by Buba *et al.* (2013) was very high, ranging from 4.50 to 6.50. Buba *et al.* (2013) stated that the pH values measured in chestnut honey are higher due to higher mineral content in chestnut honey in comparison with floral honey. Apart from that, Fallico *et al.* (2008) noticed, after analysing floral honey and chestnut honey from the area of Italy, that the pH value of chestnut honey was higher than in floral honey. Salazar *et al.* (2017) stated that the pH value of honey can be affected by the pH value of nectar and soil. The pH value of honey is an important quality parameter, because it affects the following parameters: hydroxymethylfurfural content, honey texture, and particularly the stability and the shelf life (it can indicate the occurrence of undesirable fermentation of honey).

Fiehe test is qualitative and is based on the detection of HMF that results from the dehydration of fructose, obtained by the acidic hydrolysis of sucrose. This furfurals derivative reacts with resorcinol, forming a colour. The test is considered positive when the colour is red (Almeida-Muradian *et al.*, 2013). In our study the test was negative, indicating the freshness of honey (confirmation of obtained result for HMF content), but positive Fiehe's test is never

a confirmation of of adulteration because positive result can also be the sign of aging, impurities during extraction, processing or storage. Lund's reaction is based on the precipitation of natural occurring honeys proteins by the tannic acid. The reaction is considered positive, indicating the presence of pure honey, when the precipitate varies from 0.6 to 3.0 mL (Almeida-Muradian *et al.* 2013). In our study the reaction was positive. Lugol's reaction was used to investigate starch and dextrin presence in the honey and it is based on the reaction between iodine and potassium iodide in the presence of glucose, resulting in a stained solution (red-purple to blue). The intensity of the colour depends on the amount of dextrines of glucose. The reaction is considered positive when the stained solution is blue (Almeida-Muradian *et al.*, 2013). In our study the reaction was negative, indicating absence of starch and dextrin (not naturally found in pure honey), confirming the absence of adulteration.

Table 6. Correlations (Pearson's correlation coeffcients – r) between physicochemical parameters of acacia honey

Parameter	Acidity	Electrical conductivity	Water content	HMF content	DN (Schade units)
Acidity	-	0.79*	0.71	-0.18	0.75
Electrical conductivity	-	-	0.79*	-0.20	0.88**
Water content	-	-	-	-0.49	0.75
HMF content	-	-	-		-0.42

Source: Author s' elaboration based on the obtained results
*P<0.05 **P<0.01

Correlations between different physicochemical parameters of acacia honeys are presented in Table 6. The regression analysis clearly indicated that acidity in honeys is highly correlated with electrical conductivity (0,79), diastase activity (0,75) and moisture (0,71), but negatively correlated with HMF content (-0,18). Ćirić *et al.* (2018) have found the same correlation, but their values were lower than ours. Also, the positive correlation of electrical conductivity, moisture (0,79) and distase activity (0,88, P<0,01), and negative with HMF content (-0,20) was observed. Ćirić *et al.* (2018) found strong correlation between electrical conductivity and diastase activity (0,564), but negative correlation with moisture and HMF content (-0,01 and -0,25, respectively). The positive correlation between diastase activity and moisture content can be explained by the increase of the enzyme activity in water. Lower results were found by Gomes *et al.* (2010) and Ćirić *et al.* (2018). HMF content was strongly negatively correlated with diastase activity honey (r = −0.42), what is in agreement with Ćirić *et al.* (2018) and Kędzierska-Matysek *et al.* (2016). and with moisture content (r = −0.49) what is in agreement with Ćirić *et al.* (2018).

Conclusions

Based on the results obtained by testing 19 samples of four different types of honey (floral, acacia, chestnut and forest honey) from the period between 2016 and 2019, produced in the area of the Republic of Srpska, it can be concluded that all the honey samples are in accordance with the legislation in force. The quality of the honeys was varied, based on botanical origins, and presumably, handling and storage conditions. Among the overall determined parameters diastase activity and free acidity in one honey was not acceptable according to national and international regulations. Also, all qualitative tests were negative, indicating that all samples are of natural origin, without adulteration. In the future, the quality

of honey will need to be monitored in order to improve its quality over the whole territory of Bosnia and Herzegovina.

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DYNAMICS OF SUSTAINABILITY TRANSITIONS IN THE EGYPTIAN AGRI-FOOD SYSTEM: CASE OF ORGANIC AGRICULTURE

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Abstract

The agri-food sector plays a significant socio-economic role in Egypt. Indeed, it represents the main source of income for about 40% of the population. The deterioration of the socio-economic and environmental indicators is increasing the pressure on the Egyptian agri-food system to move towards sustainability. In this context, the present review paper tackles the dynamics of transition towards sustainability in the Egyptian agri-food system using organic agriculture as a case study. The analysis was carried out through the lens of the Multi-Level Perspective on sociotechnical transitions (MLP), a major theoretical framework in transition studies, that assumes that transition comes across the interactions within and between three levels; i) the *niche*, referring to a radical innovation or a new technology, ii) the *regime*, representing the dominant system and deep sociotechnical structure, and iii) the *landscape* shown in the external factors and trends that might affect the sociotechnical system. The results show that organic farming is a growing niche in Egypt, which accounts nowadays for 2.82% of the total agriculture land, in addition of being a perfect example for targeting a sustainable agri-food system. Furthermore, the majority of the regime actors are smallholders (<1 ha) who represent 80% of Egyptian farms, with intensive mixed crop-livestock farming. The landscape factors (e.g. climate change, capitalism, population growth, revolutions, and social unrest) are expected to put pressure on the agri-food regime and to encourage the further development of organic agriculture niche, which would, in turn, increase competitive pressure on intensive agriculture. However, concerns for long-term food security, combined with limited arable land (less than 0.03 ha per capita), might help perpetuating the regime and strengthening its lock-in. Finally, this ongoing, long-term transition might follow diverse pathways to transform and reconfigure visions, policies, cultural meanings and infrastructure relating to agriculture and food in Egypt.

Keywords: *sustainability transitions, Multi-Level Perspective, agri-food system, organic farming, Egypt*.

Introduction

The deterioration of the socio-economic and environmental indicators is increasing the pressure on the agri-food system in Egypt. Indeed, 20% of the Egyptian population is below the poverty line while another 20% are near poverty, the majority of them are working in the agricultural sector (Seada et al., 2016). The loss of soil quality and agricultural land due to unsustainable practices as well as water scarcity and water pollution led to food insecurity in the Egyptian community that has reached 17.2% (13.7 million citizens) in 2011 (AbouZiyan et al., 2018). Moreover, the intensive use of chemically synthesized pesticides and fertilizers is responsible for many diseases (Durán-Lara *et al.*, 2020). Besides, poor diets and unbalanced nutrients intake have resulted in an increase of child-stunting rates to 28.9% (WHO, 2020). Furthermore, obesity among adults was 24.1% and 28.7% according to waist circumference (WC) and waist-to-hip ratio (WHR), respectively (Abolfotouh et al., 2008). These issues have

become extremely alarming thus transition to a more sustainable food system is inevitable. A sustainable agri-food system ensures food security (physical and economic access to a healthy and nutritious diet for everyone all the time) and profitability especially to poor populations (economic sustainability), enhance the resilience of the ecosystem (environmental sustainability) and incorporates a wide range of societal benefits (social sustainability) for the current and future generations (HLPE, 2014; FAO, 2018). Sustainability transition is a way to address the current unsustainable regime of production and consumption patterns, which has led to the depletion of natural resources, gas emissions, pollution, and climate change. Thus, new radical changes have to be conceptualized, explained, and implemented to transform the sociotechnical regime into a more sustainable one (Markard et al., 2012). Organic agriculture provides a good *niche* model for a sustainable system that limits and mitigates the current socio-economic and environmental issues since it helps in alleviating poverty with many successful cases in different countries (United Nations, 2002). Moreover, organic farming showed to have higher water use efficiency and a positive impact on water and soil quality (Sivaranjani and Rakshit, 2019). Besides, it contributes to the improvement of biodiversity, mitigates climatic problems, and increases the resilience of the agri-food system (Kukreja and Meredith, 2011). Despite the need for sustainability transitions in the Egyptian agri-food system, there is no research on the contribution of organic agriculture to the transition towards sustainable food systems in Egypt. Therefore, the objective of the paper is to analyse the ongoing transition towards sustainability in the Egyptian agri-food system using organic agriculture as a *niche*.

Materials and Methods

Sustainability transition is "*a fundamental change in the structure of the socio-technical regime (e.g. organizations, institutions), culture (e.g. norms, behavior) and practices (e.g. routines, skills)*" (Lachman, 2013). Since the agri-food sector is conceived as a '*socio-technical system*', it will require an authentic transition towards sustainability. A wide range of transition frameworks used to understand and support the sustainability transition (El Bilali, 2020). The *Multi-Level Perspective* (MLP) is one of the major theoretical frameworks that is used to understand transition dynamics through three socio-technical levels: *niche, socio-technical regime,* and *socio-technical landscape* (Bui et al., 2016; Geels et al., 2017). A *niche* refers to a new innovative technology, idea, or even a project with its contemporary configurations, practices, social groups, networks, norms and values, rules, policies, and regulations (Bui et al., 2016). The *socio-technical regime* describes the deep structure of the incumbent institutional system with its formal and informal rules and beliefs that have accumulated across time (Fuenfschilling and Binz, 2018). The *socio-technical landscape* refers to the external factors that affect the incumbent regime through both; slow change trends (e.g. population growth, pollution, and climate change) and exogenous shocks (e.g. wars, political and economic crises, and major accidents) over which the incumbent regime actors have minimal or no effect. The *landscape* puts pressure on the incumbent *regime* giving opportunity for the *niche* innovation to emerge (Geels et al., 2017). In this context, MLP is used to analyse the agri-food sector in Egypt and to identify challenges, levers, impacts, and outcomes of implementing organic farming on the micro-level as a locus of innovation. In order to carry out the analysis, secondary data were collected through all databases of Clarivate Analytics—Web of Science during the period from the 15th to the 20th of July 2020 - records were screened firstly by title and then by abstract according to their relevance to the topic - as well as data from grey literature (e.g., reports) and statistical data from different websites using targeted research via Google. The findings were assessed using the 'Analytical Framework to Map Sustainability Transitions in Food Systems' (El Bilali and

Probst, 2018). Hereafter, the results and discussion section describes the *landscape* factors that have shaped the current *regime* structure, followed by the regime and its interaction with organic agriculture niche.

Results and Discussion

Egypt is facing serious environmental challenges that will worsen its food security situation due to environmental and climate change threats (Farag et al., 2018). This is believed to be a result of the changes in land use and human activities during the last two decades in the Nile delta area. According to Hereher (2017), spatial changes such as transforming the agricultural land to urban land increased the land surface temperature (LST). The increase of LST resulted in a decrease in soil organic matter pool and soil fertility in the Delta area (Mohamed et al. 2019). Moreover, the use of synthetic fertilizers had the highest share of GHG emissions in Egypt at 47.2 and 45.5% for wheat and corn respectively, which are the main staple crops in the country (Farag et al., 2018). Water scarcity and water quality represent a significant threat to food production. Egypt has already reached the water poverty limit due to cropping intensity and inefficient water use (Abdelaal and Thilmany, 2019). Also, the recent infrastructure changes and dams constructions in the upstream countries, specifically Ethiopia and Sudan that are in a hunt for utilizing the Nile water for the production of food and hydropower, could harm Egypt's share of the Nile flow (Vos et al., 2019). Furthermore, the arable land in Egypt, which is less than 4% of the country's total land area, is a very scarce resource compared to the population size (Veninga and Ihle, 2018; Abdelaal and Thilmany, 2019). Land fragmentation into small units creates another challenge on production and puts pressure on smaller farms and agriculture in Egypt as it does not generate enough income and does not commercially suit the modern agriculture supply chain (Abdelaal and Thilmany, 2019; Abd-Elmabod et al., 2019). In this context, the level of poverty has significantly increased from 16.7% in 1999/2000 to 27.8% in 2015 (Abdelaal and Thilmany, 2019; Mansour, 2016). Unemployment, especially amongst youth, is high, 29% for males and 45% for females (in 2016) while women account only for 24% of the labour force (Nasr-Allah et al., 2019). The corporate agri-food system in Egypt is controlled by few firms that are growing rapidly as in 2010, 6.5% of the top food companies had a share of 41% of the total value of agricultural exports (Dixon, 2014). This is creating a sort of manipulation over the Small and Medium-Sized Export Firms (SMAEFs), that represent at least 90% of both Egyptian farmers and export corporations (Hatab and Hess, 2013; Dixon, 2019). Moreover, globalization of agri-food value chains and the liberalization of agricultural trade enhanced the exchange of agricultural and food commodities (Hatab et al., 2019). But it also increased the *landscape* of food safety and quality standards (FSQSs) for health and environmental concerns, which raised the norms to levels that many developing countries cannot fulfil (Swinnen and Vandemoortele, 2011). Furthermore, the social instability that was noticed during what's so-called the *Arab Spring* as a result of various landscape elements; corruption, privatization, capitalism, and falling the country in the hands of crony elites who shaped the economic and industrial policies for their favour other than the public interest (El-Haddad, 2020). In addition, the increasing food prices, poor living standards and health systems, environmental degradation, and deterioration of the educational quality that were a further evidence of governmental failures and abandonment of state responsibilities (Mansour, 2016). These factors are increasing the economic pressure and threatening Egypt's capacity to achieve long-run food security.

The overall Egyptian food self-sufficiency is 88% and for cereals is 66.04%, while the total cultivated land area is 3.745 Million ha and the total renewable water resources are 58.3 Bil m^3/yr (Bagnied and Speece, 2019). During the last three decades (1984–2015), cropland area had increased by 33.7%, with an annual average increase of 31,000 ha (Xu et al., 2017). This

expansion on the socio-ecological spaces and the intensification of agriculture and food in 'reclaimed land' allowed Egypt to introduce high-value agriculture commodities in the local and export markets (Dixon, 2015). The food sector in Egypt is one of the top five sectors with a high absorption capacity as it has strong linkages with the other sectors and a great impact on the Egyptian economy (El Mekki et al., 2015). Egyptian agri-food commodities have always been directed towards the European Union (EU), representing about one-third of the country's agri-food exports within the period extending from 2000 to 2016 (WITS, 2017). However, Egypt's FSQSs are considered the strongest barrier that's hindering the exportation of food commodities especially for SMAEFs (Hatab et al., 2019).

Wheat is the main ingredient in the Egyptian diet and provides 60% of the nation's daily calorific and protein intake (Tadesse et al., 2017). It is heavily subsidized in the country and the general availability of cheap wheat flour creates a very high domestic demand for wheat (Veninga and Ihle, 2018). Egypt exhibits extreme dependency on wheat yields, as it was ranked the third world wheat importer in 2004 and 2008, and the second in 2014 (Dong et al., 2018). Egypt's high dependence on wheat imports from other countries for ensuring food security makes the country vulnerable to increasing food prices and other shocks to the world market, which might challenge flows of imports (Veninga and Ihle, 2018; Abdelaal and Thilmany, 2019). Import dependency is increasing despite Egypt's efforts to attain self-sufficiency in the main crops and achieve food security through maximizing water use efficiency and encouraging the sustainable use of land and water resources (Abdelaal and Thilmany, 2019). The regime still does not have a comprehensive strategy for sustainable development, and policy progress on environmental sustainability is very slow (Mansour, 2016). The cultural changes promoted by the development of the corporate agri-food system reinforced two main national dietary trends: increase in animal protein consumption and inadequate caloric intake within some societal class, and the widening of access to affordable food across another class associated with a shift in diet towards foods higher in fats and sugars (Galal, 2002). This space of dietary convergence also helps to explain the disease prevalence among the wealthy and the poor (Dixon, 2019). Indeed, the spread of diseases such as Cardiovascular Diseases (CVD) and cancer, due to the unhealthy lifestyle and poor dietary intake, has become very popular in Egypt (Jawaldeh and Al-Jawaldeh, 2018).

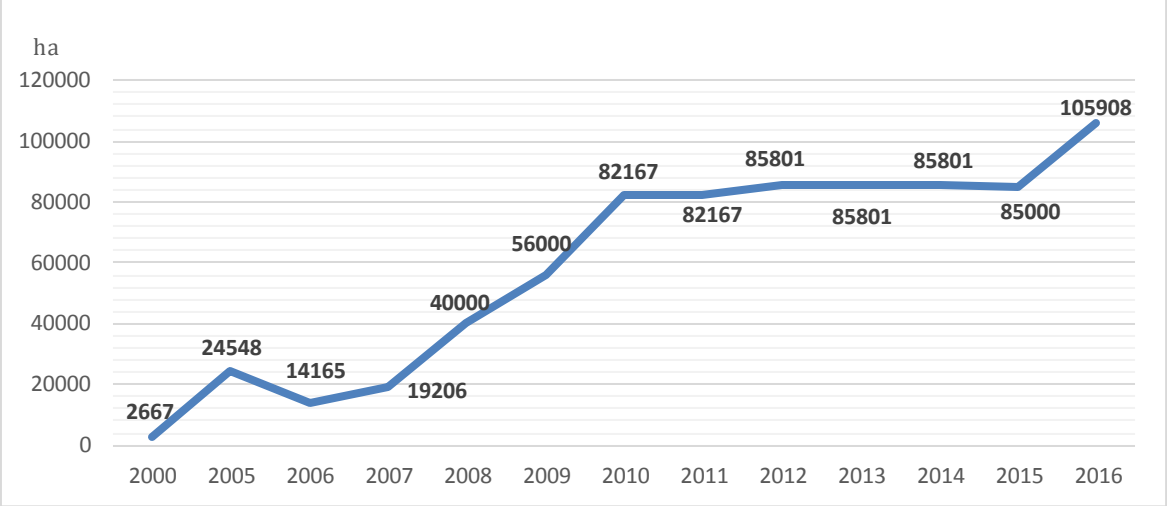
Sustainability transition is characterized by being a multi-dimensional and multi-actor process; since sociotechnical systems are a range of different elements, networks, social groups, and agencies that are interdependent (not linear) with co-evolutionary processes. Such transformation is called 'sociotechnical transition' because it does not consider only the technological modifications but also cultural and consumer practices, policies, and infrastructure foundations (Köhler et al., 2017). Organic agriculture is considered an answer to the food system crises and the environmental impacts as a low input and eco-friendly technique (Sacchi et al., 2018). Organic agriculture provides an alternative approach for the sustainable agri-food system since its policies and practices go hand in hand with the core principles of sustainable agriculture and sustainable agri-food systems (Strassner et al., 2015). The organic movement in Egypt was initiated in 1976 as a bottom-up approach by the visionary Dr. Ibrahim Abouleish who built the first biodynamic farm according to the concepts we know today. Starting from 70 ha of arid desert soil in Sharkia governorate it was expanded to reach 1,390 ha in 2018 (Northeast of the Nile Delta) (SEKEM, 2018). Organic land area in Egypt is 105,907 ha, which contributes with 2.8% of the world's total organic land area, and consists of 970 organic farms (Siam and Abdelhakim, 2019).

A profound sustainability transition involves a reconfiguration of the related *regimes*. To detect possible levers for inducing changes, a deep look into the *niche-regime* interactions is required (El Bilali and Probst, 2018). The Egyptian *sociotechnical regime* is experienced with trade and exportation of food, thanks to the premium price of organic agriculture products,

availability of organic production inputs (e.g. compost, biological control products), and the low labour cost. Moreover, the ability of organic agriculture to mitigate the environmental problems arising in Egypt such as land degradation, water scarcity and pollution, gives organic farming a competitive advantage over its conventional counterpart (Sadek and Shelaby, 2011; Rahman et al., 2020). The certification and inspection procedures are carried out by foreign certification bodies according to different international standards mainly the European Union regulations and the National Organic Plan (NOP) of the United States (Suzana et al., 2019). These privileges allow Egypt to export organic products especially with the increasing demand for organic in more than 160 countries and the diversified production of Egyptian fruits and vegetables due to favourable climatic conditions (Sadek and Shelaby, 2011; Rahman et al., 2020). This permitted organic agriculture to grow annually with a rate of 18.18%, as illustrated in Figure 1. Unfortunately, these privileges are devoted to capitalists and large investors, rather than small and medium enterprises, mainly due to the high certification cost, the low performance of extension services and the small farm size. The vast initial cost of production of organic products gives it a higher price and makes it hard for the majority of Egyptians to afford since a large segment of the community is poor (Sadek and Shelaby 2011). Despite, the growing public awareness and the increasing number of NGOs who are lobbying for organic agriculture, the incumbent conventional producers and capitalists are against the development of the new niche (Vincenti, 2016). Moreover, the social instability and political unrest is keeping the sociotechnical regime busy handling pressing socio-economic problems thus delaying the sustainable innovation progress.

The presence of agricultural institutions and exportation infrastructure is generally inadequate in terms of transportation facilities, roads, ports, storage, and airports, and particularly poor in rural areas (Bagnied and Speece, 2019). Institutions and supply chains are generally very inefficient, with much bureaucracy, corruption, poor administration, and insufficient facilities and equipment (Bagnied and Speece, 2019). Many laws and regulations are old-fashioned and led to inadequacies in production and distribution. Indeed, these laws resulted in land fragmentation and dependency on governmental support through subsidies. Moreover, laws concerning safety, environment, marketing and consumer protection are either lacking or inadequate. They are imposing an adverse effect on production and they need reassessment and updating (Bagnied and Speece, 2019).

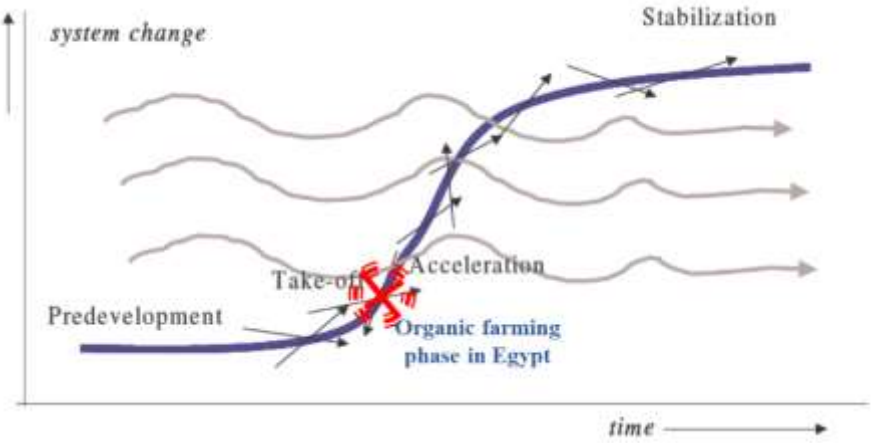
Figure 1. Evolution of organic land area in Egypt, 2000-2016.



Source: Willer and Lernoud (2018).

The recently introduced national organic regulation in January 2020 could be a good opportunity for the growth of the domestic market. All in all, the organic farming sector in Egypt is at its infancy stage heading towards the take of phase of transition as shown in Figure 2 (Siam and Abdelhakim, 2019), where different processes and stimulants are building up and reinforcing the organic farming niche to emerge.

Figure 2. Stage of organic agriculture development in Egypt.



Source: Adapted from Loorbach and Rotmans (2006).

Conclusion

The Egyptian food self-sufficiency is at great risk due to insufficient water and land capacity to satisfy the need of a rapidly growing population, thus the need for a transition to a more sustainable system is a vital requisite. Throughout this study, organic farming is proposed as an innovation that will mitigate landscape problems and enhance the performance of the agri-food sector in Egypt. Nevertheless, the review shows that organic farming is a slow-growing niche in Egypt that necessitates fundamental changes within the regime policies and infrastructure; this can be achieved through multi-stakeholder dialogue and consultation at all levels and with the active participation of the private sector, that's important for the gradual alignment and harmonization of responsive policy changes in the agri-food sector. Also networking between the specialists and experts, developing research and improving extension intervention and agricultural technological progress are crucial in the sustainability transition journey. Meanwhile, educating and increasing public awareness about the environmental, nutritional, and dietary habits is fundamental for the alleviation of health problems and attaining food security. The development of the organic agriculture niche is vital to foster sustainability transitions in the Egyptian agri-food system, which is, in turn, paramount not only to achieve the country's long-term food and nutrition security but also to contribute to climate change mitigation and the achievement of the Sustainable Development Goals (SDGs) in line with the Egypt's Sustainable Development Strategy and Vision 2030.

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INFLUENCE OF STORAGE TEMPERATURE ON THE DEVELOPMENT OF YEASTS IN FRUIT JAM WITH POTASSIUM SORBATE

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Abstract

The aim of our work was to study the change in the quantity of yeast in unsterilized fruit jam with potassium sorbate, packaged in soft packs of the "Doy Pack" type, at different storage temperatures to establish shelf life. The objects of research were samples of blueberry jam, canned by hot bottling using potassium sorbate and packaged in soft packs of the "Doy Pack" type. The *Saccharomyces cerevisiae* and *Candida scottii* strains were used. Jam samples infected with test microorganisms were placed in a refrigerator (6±1.0°C) and in a thermostat (24±0.5°C). The control samples of jam in the original packaging were also stored at the same temperatures. Sampling of contaminated products was carried out every 3-4 days for 28 days, then every 2 weeks. Control samples of jam were examined monthly. In addition, organoleptic and physicochemical parameters of control and infected samples were determined. As a result of studies, it was found that the industrial sterility of the control samples of jam remained during 6 months of storage at temperatures of 6°C and 24°C. Organoleptic and physicochemical parameters of control samples of product during storage did not change compared to their initial value. The storage of yeast contaminated samples at different temperatures indicated the absence of favorable conditions for the development of microorganisms. At a storage temperature of 24°C, all test microorganisms died after 25 days of storage. Visible changes in the organoleptic characteristics of contaminated samples were not detected. After the 4 months of storage at a temperature of 6°C, contaminated samples contained yeast in the amount of some tens of CFU/cm^3. The data obtained suggest that canned blueberry jam by hot bottling using potassium sorbate, packaged in a soft pack "Doy Pack" type, is resistant to microbiological spoilage and can be stored for at least 6 months under standard conditions.

Keywords: *Jam, Storage, Yeast, Survival*

Introduction

Yeast is usually a minor component of the microbiota and cannot compete with bacteria in most food systems. But in the presence of certain factors (low pH, low values of water activity, high sugar content, etc.) yeast can become the main cause of food spoilage [Blackburn, 2008]. Fruit jams are a good breeding ground for yeast that can cause spoilage. Such yeasts include: *Saccharomyces cerevisiae* and some types of yeast of the genus *Candida* [Blackburn, 2008]. According to available data [Blackburn, 2008], yeast of the genus *Saccharomyces* grows well at temperatures between 15 and 30°C. At temperatures below 10°C, the growth rate is significantly reduced, and the lag phase becomes longer. The lowest temperature at which the growth of these yeasts stops is not exactly known, but there is evidence of the growth of *S. cerevisiae*, *S. bayanus*, *S. unisporus*, *S. dairienensis* and *S. exiguus* at 4–7°C for 1–3 weeks [Deak, Beuchat, 1993; Drocklenhurst, White, Dennis, 1983; Jermini, Schmidt-Lorenz, 1987; Savard *et al.*, 2002]. The minimum growth temperature depends on factors such as pH value, sugar and salt concentration [Betts, Linton, Betteridge,

2000; Fleet, 1992]. The yeast of the genus *Saccharomyces* ceases to develop at a_w below 0.85–0.88 [Tokuoka, 1993; Lagos, Silva-Graca, Lucas, 1999]. The minimum a_w for yeast growth depends not only on the sugar and salt content, but also on the type of solute. In addition, the upper limits of growth in the presence of other stress factors (low pH value, low temperature, the presence of antibacterial drugs) are significantly reduced [Tibury, 1980]. There are few systemic comparative studies on the effect of pH values, weak organic and inorganic acids, often used as preservatives, on the growth of yeast of the genus *Saccharomyces* [Blackburn, 2008]. The optimal conditions for the growth of yeast of the genus *Saccharomyces* are acidic conditions (pH 3.0–7.0), and the limiting conditions are pH 1.5–2.5 and pH 8.0–8.5 [Betts, Linton, Betteridge, 2000; Praphailong, Fleet, 1997]. Some minimum concentrations that inhibit *S. cerevisiae* at pH 3.5 are given in [Fleet, 1992; Praphailong, Fleet, 1997]. According to these authors, the minimum inhibitory concentration of sorbic acid ranges from 200 to 600 mg/l. According to the literature [Blackburn, 2008], yeasts of the genus *Candida* make up almost 25% of all known yeasts, therefore, the influence of environmental factors and their stress effect, as well as the resistance of this yeast group to the effects of these factors, is the same as for the entire group yeast. Temperature ranges for the growth of yeast of the genus *Candida* are in the range from 0 to 40°C. Many species are mesophilic and grow best at temperatures between 25 and 35°C. The growth temperature may be less than 37°C (e.g. *C. zeylanoides*, *C. vini*) or may exceed 45°C (e.g. *C. albicans*, *C. glabrata*). The lower temperature limit for growth can be several degrees below zero, provided that the solutes are in an unfrozen state, as in many food systems. Another factor is the availability of free water, i.e. the value of a_w. Most *Candida* yeasts grow well at a_w from 0.90 to 0.95, but survive at lower values (about 0.80) due to the increased concentration of solutions. Factors such as the type of solution, temperature, pH value, and other factors influence the resistance of *Candida* yeast to low water activity values [Tokouka, 1993].
The factor limiting the growth of yeast, including the genus *Candida*, is the pH value. *Candida* generally prefer a slightly acidic environment and grow optimally between pH 4.5 and 5.5. At the same time, the facts of growth of yeast of the genus *Candida* (*C. krusei* and *C. valida*) are known even at pH 1.5. Weak organic (benzoic and sorbic) acids used as preservatives can effectively inhibit yeast growth if applied in appropriate concentrations and if the pH values are low enough to slow down the dissociation of these weak acids [Blackburn, 2008]. Concluding a brief review of the properties and role of yeasts of the genus *Saccharomyces* and *Candida* in food spoilage, it can be noted that, despite numerous studies, the issues of their survival in unsterilized fruit jams with a preservative remain insufficiently studied, which was the purpose of this work.

Material and Methods

The object of the study was pilot samples of non-sterilized hot bottling blueberry jam with potassium sorbate (Tables 1 and 2). The subject of research is the strains of *Saccharomyces cerevisiae* and *Candida scottii*, kindly provided to us from the working collection of microorganisms of the Department of Biotechnology of the Belarusian State Technological University. For experimental studies, selected samples of blueberry jam were divided into several parts. The first and second parts were stored at a temperature of 6±1.0°C (refrigerator) and 24±0.5°C (thermostat XT-3/70-1). The third part in the form of a combined sample was poured into four sterile glass jars (type III-82-450) and inoculated with a suspension of cells of test yeast strains (1 cm^3 each), than mixed thoroughly to uniformly distribute the yeast over the product volume. Contaminated jams (2 jars with each microorganism) were placed in a refrigerator (T = 6±1.0°C) and in a thermostat (T = 24±0.5°C). The indicated temperature modes were selected based on the following considerations: 6±1.0°C is the temperature at

which the typical microbiota of fruit jams does not develop; 24±0.5°C is optimum temperature for yeast growth.

Table 1 – Identification signs of the research object

Identification signs	Description
Designation of the documentation for which the products are manufactured	TC BY 291416503.004-2019 (draft of specification)
Manufacturing date	14.11.2019
Type of packaging	Soft packaging «Doy Pack»
Net weight of packing unit, kg	0,3
Component composition	Frozen or fresh blueberries, sugar, citric acid, pectin, potassium sorbate

Table 2 – Summary characteristics of the research object

Research object	Physical and chemical indicators			
	pH	Mass fraction of soluble solids, %	a_w	Eh, mV
Unsterilized hot bottling blueberry jam with potassium sorbate	2,80	69,84	0,685	255,0

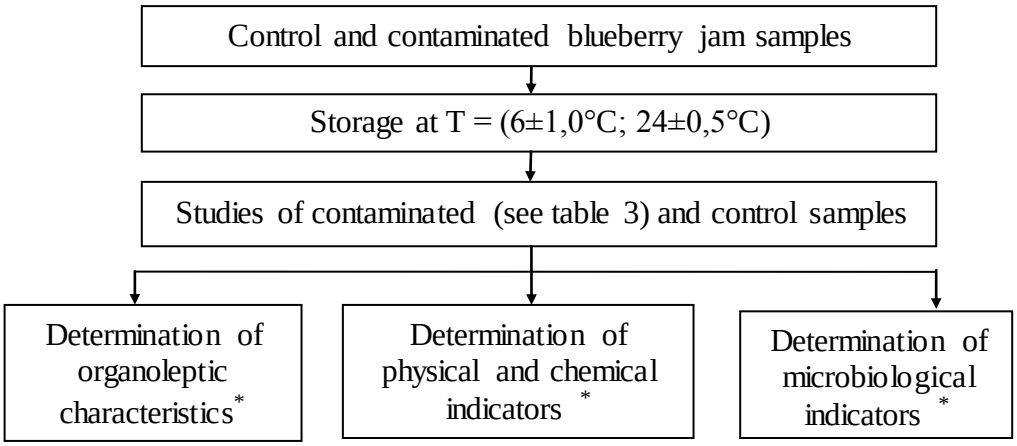
In the experiments, we used 25-day-old cultures of the studied test strains grown on slant Sabouraud agar at a temperature of 24±0.5°C. The research scheme and the experiment plan are shown in Figure 1 and Table 3, respectively.

Table 3 – Experimental plan for determining the survival of test microorganisms in unsterilized blueberry hot bottling jam with potassium sorbate

Storage temperature, ºC	Test strain designation and initial content in the contaminated product, CFU / cm^3	Frequency of product sampling	Thermostating conditions
6	*Saccharomyces cerevisiae,* $2,5\times10^5$ *Candida scottii,* $1,7\times10^5$	Every 3–4 days for 28 days, then every 2 weeks for 5 months	Sabouraud agar, 24±0,5°C, 120 h
24	*Saccharomyces cerevisiae,* $4,3\times10^5$ *Candida scottii,* $1,7\times10^5$		

The following indicators were determined in the contaminated and control samples of jam using standardized methods or by the instrument manual:
– appearance and consistency (according to GOST 8756.1-79);
– water activity (according to GOST ISO 21807-2015 using the water activity analyzer «Roremeter RM-10», the measurement error is ± 0.02);
– hydrogen indicator (pH) (according to GOST 26188-84 using a pH meter ionomer «Hanna Instruments HI 2211-02», the measurement error is ± 0.01);

– the content of soluble solids (by the refractometric method according to GOST ISO 2173-2013 using the refractometer «Atago NAR-1T», the measurement error is ± 0.1%);
– redox potential (Eh) (the following measuring systems were used: ionomer I-160 M, high-temperature platinum electrode EVP-1 and silver chloride reference electrode EVL-1M3.1, error Δ (Eh) ± 3 mV).



Note. * Organoleptic and physicochemical indicators of contaminated jam samples were determined at the end of the experiment and organoleptic, physicochemical and microbiological indicators of control samples were detected monthly.

Figure 1 – Scheme of studies to determine the dynamics of the number of test strains of yeast in blueberry jam unsterilized hot bottling with potassium sorbate during storage at different temperature conditions

In control samples of jams, the following microbiological parameters were determined:
– the presence and quantity of mesophilic aerobic, facultative anaerobic and anaerobic microorganisms (according to GOST 30425-97);
– the amount of yeast and molds (according to GOST 10444.12-2013);
– the presence of bacteria of the group of Escherichia coli (coliform bacteria) in 1 cm^3 of the product (according to GOST 31747-2012).
The processing of the results of microbiological studies was carried out using the methods of mathematical statistics [Garnayev, 1999; Alekseev, Chesnokova, Rudchenko, 2008].

Results and Discussion

The results of studies of changes in the amount of yeasts of the species *S. cerevisia* and *C. scottii* in blueberry jam during storage at different temperatures are shown in Figures 2–5, from which it can be seen that during storage of contaminated samples of blueberry jam, a gradual dying off of test microorganisms occurred. The rate of death of the yeast depended on the temperature conditions of product storage. Despite the fact that the temperature of 24±0.5°C is optimal for the development of these types of microorganisms, the test strains of *S. cerevisiae* and *C. scottii* died in blueberry jam on the 25th day of storage (Figures 2 and 4).
The treatment of experimental data (see the equations shown in Figures 2 and 4) using polynomial approximation indicated that the obtained dependences of the number of test microorganisms on the storage duration showed good convergence of curves and experimental data (R^2 were equal to 0.971 and 0, 9658, respectively) and can be used to

predict the activity of *S. cerevisiae* and *C. scottii* yeasts in fruit jams with a high sugar content and with sorbic acid under normal storage conditions.

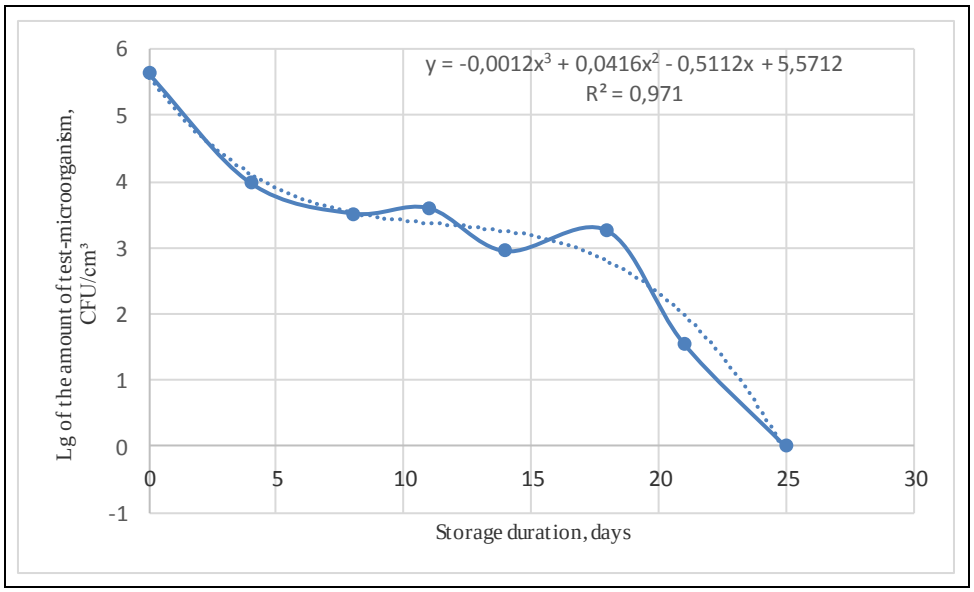


Figure 2 – Survival curve of *S. cerevisiae* in blueberry jam stored at 24±0.5°C

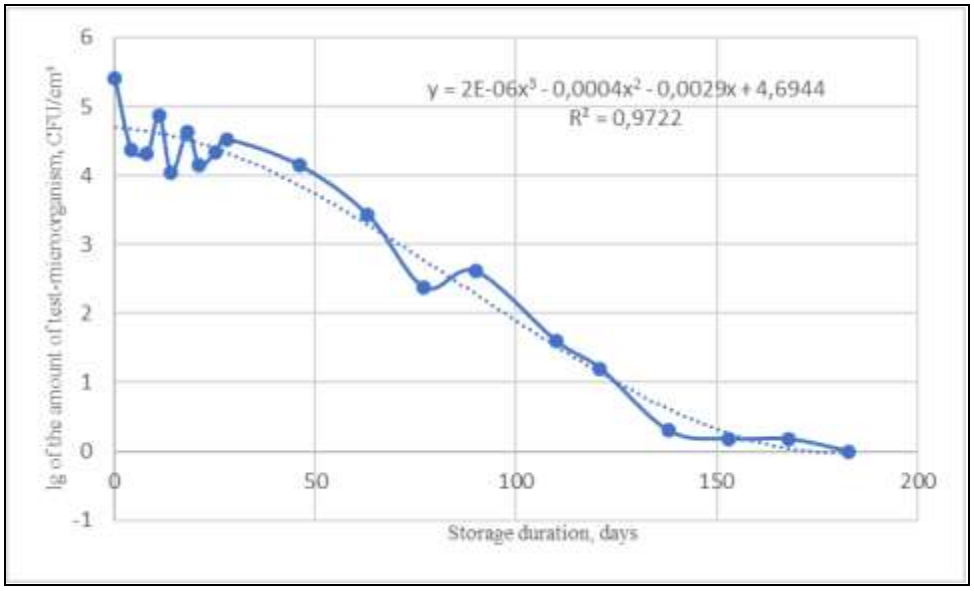


Figure 3 - Survival curve of *S. cerevisiae* in blueberry jam stored at 6±1.0°C

Other results were obtained when examining samples of blueberry jam with sorbic acid, contaminated with yeast test strains and stored in a refrigerator (Figures 3 and 5). First, the survival time of both strains in blueberry jam was six months. Second, when comparing the dynamics of the number of test strains during the first month of storage at different temperatures, a significant difference in the rate of die-off is obvious: in a refrigerator, the number of test microorganisms decreased on average by only 1 order of magnitude. The use of polynomial approximation for treatment data on the survival of test yeast strains in blueberry jam stored at 6±1°C (see equations in Figures 3 and 5) allowed to obtain an acceptable coincidence of the curves with the experimental data (R^2 = 0.9722 and R^2 = 0 ,

9487). Thus, our data confirm the fact that yeast can adapt to a certain extent and survive in unfavorable environmental conditions.

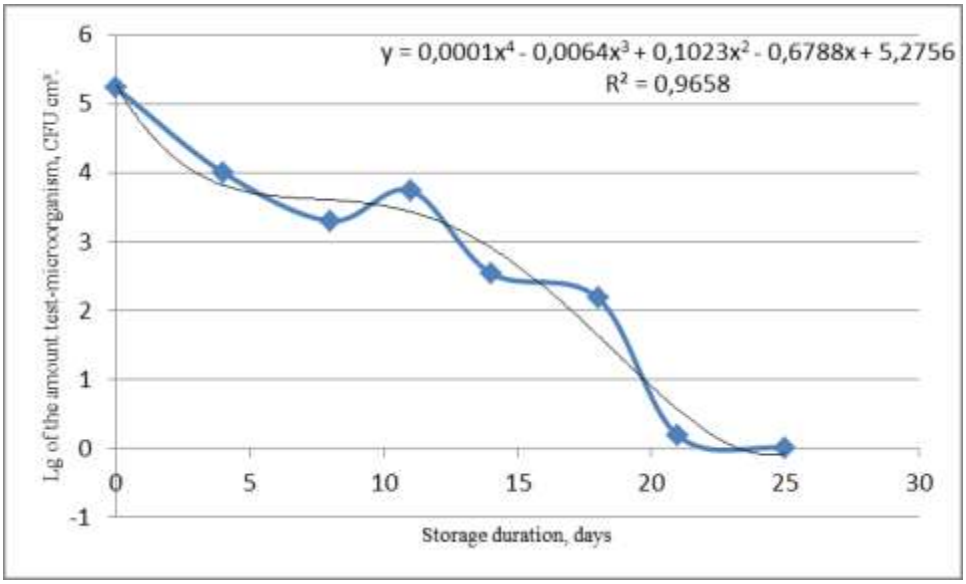


Figure 4 – Survival curve of *C. scottii* in blueberry jam stored at 24±0.5°C

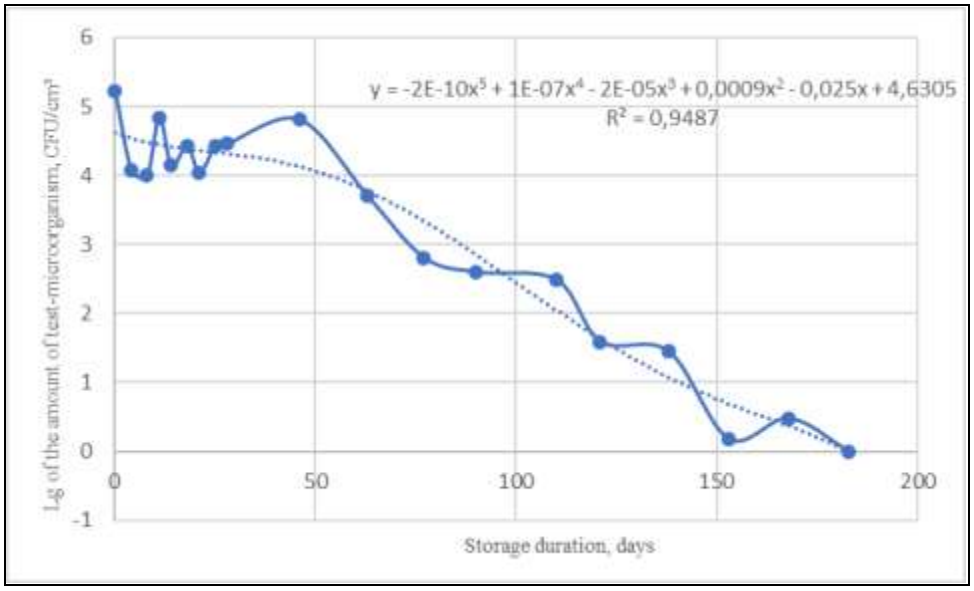


Figure 5 – Survival curve of *C. scottii* in blueberry jam stored at 6±1.0°C

The results of physicochemical tests of contaminated jam samples and their comparative analysis are shown in Tables 4 and 5. The data given in tables 4 and 5 indicate some influence of yeast on the physicochemical parameters of contaminated samples of blueberry jam with potassium sorbate. At the same time, the *C. scottii* strain exhibited greater enzymatic activity compared to the *S. cerevisiae* strain during short-term storage at a temperature of 24±0.5°C. It should also be noted that the presence of test yeast strains had a greater effect on such indicators of blueberry jam as water activity (all storage temperatures) and Eh (at a storage temperature of 24±0.5°C). However, no visible signs of spoilage, i.e. changes in organoleptic indicators (appearance and consistency) of contaminated samples of blueberry jam, were found.

Table 4 – Physicochemical indicators of control and contaminated samples of blueberry jam after the end of the experiment

Test microorganism	Storage temperature, °C	Storage duration, days	Mass fraction of soluble solids, %	pH	a_w	Eh, mV
S. cerevisiae	6±1,0	183	67,60	3,02	0,666	220,2
C. scottii			67,20	3,03	0,699	220,8
Control			68,90	2,98	0,722	223,1
S. cerevisiae	24±0,5	25	68,95	2,86	0,633	245,5
C. scottii			68,35	2,98	0,624	242,5
Control			69,50	2,86	0,660	258,5

Table 5 – Comparative analysis of physicochemical parameters of control and contaminated samples of blueberry jam after completion of the experiment

Test microorganism	Storage temperature, °C	Storage duration, days	Change (in %) to control			
			Mass fraction of soluble solids	pH	a_w	Eh
S. cerevisiae	6±1,0	183	–1,89	+1,34	–7,76	–1,30
C. scottii			–1,72	+1,68	–3,19	–1,04
S. cerevisiae	24±0,5	25	–0,80	0	–4,10	–5,03
C. scottii			–1,66	+4,20	–5,46	–6,19

To confirm that the control samples of blueberry jam are resistant to spoilage, we conducted microbiological studies indicating the absence of mesophilic aerobic, facultative anaerobic and anaerobic bacteria, yeast, molds and coliform bacteria in 1 cm^3 of jam samples.

Conclusions

Our research results allow us to formulate the following conclusions. Unsterilized hot bottling blueberry jam with potassium sorbate according to physicochemical parameters refers to products with a minimal risk of yeast development: the value of water activity is below 0.7; pH value does not exceed 3.0; the content of soluble solids is more than 65%. Storage of samples of blueberry jam contaminated with *S. cerevisiae* and *C. scottii* at different temperatures indicated the absence of favorable conditions for yeast development. At storage temperatures equal to 24±0.5°C and 6±1.0°C, all test microorganisms died after 25 and 183 days of storage, respectively. No visible signs of spoilage (change in consistency, appearance and color, gassing) of contaminated blueberry jam samples stored at different temperatures were found. Thus, the use of preservatives and short-term heat treatment (hot bottling) in the production of fruit jams allows, to a greater extent, in comparison with classical technology, to preserve natural properties without loss of functional qualities, ensuring a long shelf life, stability, safety and organoleptic acceptability. However, because food ecosystems are rarely static [Blackburn, 2008], there is opportunity for adaptation and survival of certain yeast strains in unfavourable environments, such as fruit jams with potassium sorbate.

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HPLC ANALYSIS AND ANTIMICROBIAL POTENTIAL OF PLANT EXTRACTS

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Abstract

The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine. The therapeutic effect of these plants is related to the content of many biologically active compounds, including flavonoids, phenols and tannins. Although there were several hundred thousand plant species around the globe, only a small proportion has been investigated both phytochemically and pharmacologically. Therefore, the aim of this study was to determine polyphenolic compounds and antimicrobial potential of acetone extract of *Echium italicum* and *Anchusa officinalis*. The plants belong to the family *Boraginaceae*, and have long been used in folk medicine. Determination of polyphenolic compounds in tested extracts was performed by HPLC analysis. The antimicrobial potential of acetone extracts was examined by the microdilution method. HPLC analysis of examined extracts of *A. officinalis* and *E. italicum* confirmed the presence of the following polyphenolic compounds: p-hydroxybenzoic acid, chlorogenic acid, p-coumaric acid, ferulic acid, sinapinic acid, rutin, lutein glycoside, apigenin glycoside, rosmarinic acid, quercetin, lutein, naringenin and kaempferol. Acetone extracts of the tested plants showed extremely good antimicrobial potential compared to the standard antibiotic amracin. The maximum antimicrobial activity showed by the acetone extract of *E. italicum* in the control of *S. enteritidis* (3.91 μg/ml) and *P. vulgaris* (7.81 μg/ml), and acetone extract of *A. officinalis* in the control of *E. aerogenes* and *L. ivanovii* (31.25 μg/ml). This study showed that acetone extracts had a significant amount of polyphenolic compounds with extremely good antimicrobial potential.

Keywords: *HPLC analysis, Antimicrobial potential, Plant, extract.*

Introduction

Before the advent of modern medicine, people depended essentially of the plants for the treatment of various diseases, and they still have been an important source for the development of new drugs (Kuete *et al.* 2009). The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine (Dubey *et al.* 2011). According to the WHO, between 65% and 80% of the populations of developing countries currently use medicinal plants as remedies (WHO, 2011). The therapeutic effect of these plants is related to the content of many biologically active compounds, including naphthaquinones, flavonoids, terpenoids and phenols (Sharma *et al.* 2009). The advantage of herbal reparations over synthetic drugs is reflected in the fact that they are available to us in nature and are less toxic. To guarantee the quality of herbal medicines, certain steps established in the Pharmacopoeias must be followed, including correct identification of the plant species, analysis of the purity and confirmation of the presence and minimum concentration of the active ingredients (Brandao *et al.* 2013). The resistance of microorganisms to antimicrobial drugs has become a problem of global importance. Of the 2 million people who acquire bacterial infections in US hospitals each year, 70% of cases now involve strains that are resistant to at least one drug (IDSA,

2004). For this reason, there is a need to find new antimicrobial compounds from alternative sources, such as plants. According to Amenu (2014) medicinal plants possess immunomodulatory and antioxidant properties, leading to antibacterial activities. *Echium italicum* and *Anchusa officinalis* belong to the *Boraginaceae* family. Numerous studies have confirmed their use in folk medicine (Khare, 2007; Altundag and Ozturk, 2011). Many members of the *Boraginaceae* family produce secondary metabolites such as alkaloids, naphthoquinones, polyphenols, phytosterols and terpenoids (Gottschling et al, 2001; Zhou and Duan, 2005), which possess a wide range of pharmaceutical activities. Therefore, the aim of this research was to the determine polyphenolic compounds and antimicrobial activity of acetone extract of *Echium italicum* and *Anchusa officinalis*.

Matherial and methods

Chemicals

Muller–Hinton broth (MHB), resazurin, amracin, protocatechuic acid, caffeic acid, vanillic acid, chlorogenic acid, syringic acid, ferulic acid, rutin, rosmarinic acid, naringenin, luteolin, kaempferol and apigenin were purchased from Sigma- Aldrich GmbH (Sternheim, Germany). Methanol, acetone, quercetin and formic acid (standards for HPLC) were supplied by Merck KgaA (Darmstadt, Germany).

Plant material and extracts preparation

The plants were collected in the flowering stage of development in Serbia . Plant material was dried at room temperature. The above-ground part of the plants was grinded by cylindrical crusher and extracted with acetone in Soxhlet apparatus. The solutions were evaporated on a rotary-evaporator at a temperature of 40 °C.

HPLC screening

The HPLC analysis of acetone extracts were performed on the HPLC instrument Agilent 1200 Series with UV-Vis DAD for the detection of multi wavelengths. Separation of samples was performed using an Agilent column, Eclipse XDB-C18 (4.6 x 50 mm, 1.8 μm), which was thermostated at 25^0C. Two solvents were used for the gradient elution: A (H_2O+2% HCOOH) and B (80% ACN+2% HCOOH+H_2O). The identification of the phenolic compounds was done by comparing its retention time of the original standard.

An antimicrobial activity

Antimicrobial activity of the acetone extracts were performed by microdilution method (CLSI, 2012). In the study were included pure cultures of the following bacteria: *Proteus vulgaris* ATCC 13315, *Salmonella enteritidis* ATCC 13076, *Enterobacter aerogenes* ATCC 13048, *Salmonella Typhimurium* ATCC 14028 and *Listeria ivanovii* ATCC 19119. The extract solutions were serially diluted (1:1) in Mueller–Hinton Broth in a series of 96-well microplate wells, and then bacterial culture was added at an approximate concentration of 1.5 × 10^8 CFU/ml (colony-forming units). And then the resazurin indicator was added to all the holes. The microplates were incubated for 24 h at 37 °C, and thereafter minimum inhibitory concentration (MIC) was determined.

Result and discussion

The most striking feature of natural products in connection to their long lasting importance in drug discovery is their structural diversity that is still largely untapped (Veeresham, 2012). Although there are several hundred thousand plant species around the globe, only a small

proportion has been investigated both phytochemically and pharmacologically (Boligon and Athayde, 2014). To isolate and purify the plant based products like secondary metabolites and proteins HPLC is highly useful for researchers and industrialists those who focus on quality (Thirumal and Laavu, 2017). HPLC analysis of examined extracts of *A. officinalis* and *E. italicum* confirmed the presence of the following polyphenolic compounds: p-hydroxybenzoic acid, chlorogenic acid, p-coumaric acid, ferulic acid, sinapinic acid, rutin, lutein glycoside, apigenin glycoside, rosmarinic acid, quercetin, lutein, naringenin and kaempferol (Table 1). The most common compounds in the acetone extract of *Anchusa officinalis* were rosmarinic acid (58,961 mg/g), chlorogenic acid (4,646 mg/g), naringenin (3,142 mg/g), luteolin glycoside (6,775 mg/g) and rutin (2,073 mg/g) – fig.1, which is in accordance with the research of Zengin et al (2015). Numerous studies have confirmed the antioxidant, antimicrobial, antiviral, antidiabetic, and anticarcinogenic properties of rosmarinic acid and rutin (Petersen and Simmonds, 2003; Lacopini *et al.* 2008; Hooker *et al.* 2001; Huang and Zeng, 2006).

Table 1. HPLC data analysis of acetone extract of plant *E. italicum* and *A. officinalis* (mg/g)

Compound/plant (mg/g)	*Echium italicum*	*Anchusa officinalis*
Protocatehuic acid	n.d.	n.d.
p-Hydroxybenzoic acid	4.865	0.845
Caffeic acid	n.d.	n.d.
Vanillic acid	n.d.	n.d.
Chlorogenic acid	1.915	4.646
Syringic acid	n.d.	n.d.
p-Coumaric acid	n.d.	0.008
Ferulic acid	0.519	0.247
Synapic acid	n.d.	2.057
Rutin	4.926	2.073
Luteolin-glycine	0.748	6.775
Apigenin-glycine	n.d.	1.605
Rosmarinic acid	12.131	58.961
Quercetin	1.006	0.396
Luteolin	n.d.	n.d.
Naringenin	0.371	3.142
Caempherol	0.985	0.417
Apigenin	n.d.	n.d.
Σ	27.466	81.172

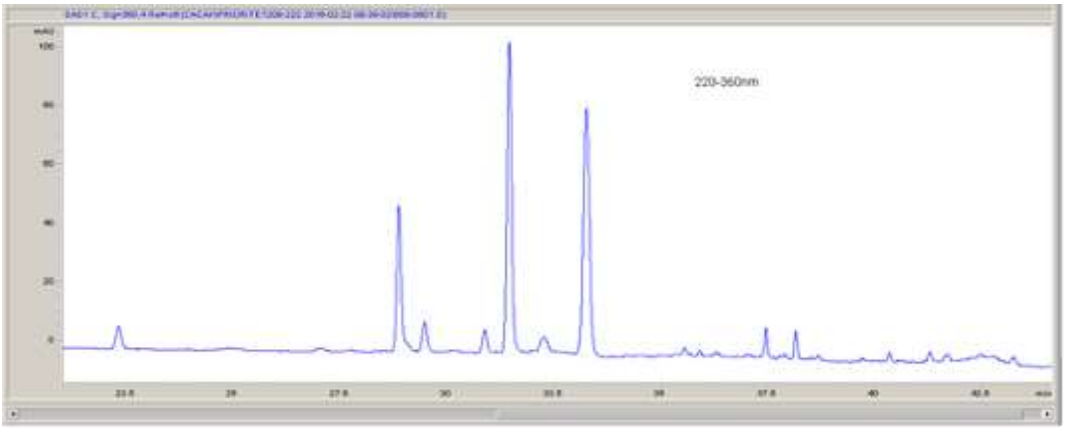


Fig. 1. HPLC chromatogram of acetone extract of *Anchusa officinalis* L.

The most dominant compounds in acetone extract of *Echium italicum* (Fig. 2) were rosmarinic acid (12,131 mg/), rutin (4,926 mg/g) and p-hydroxyenzoic acid (4,865 mg/g), which is partly in agreement with the results of Dresler *et al.* (2017). p-Hydroxybenzoic acid showed antimicrobial, antifungal, antisickling, and estrogenic activities (Pugazhendhi *et al.* 2005; Chong *et al.* 2009). According to Horvath *et al.* (2007) p-Hydroxy benzoic acid increases abiotic stress tolerance of plant and also increases the impermeability of the cell wall, leading to increased resistance against pathogen infection.

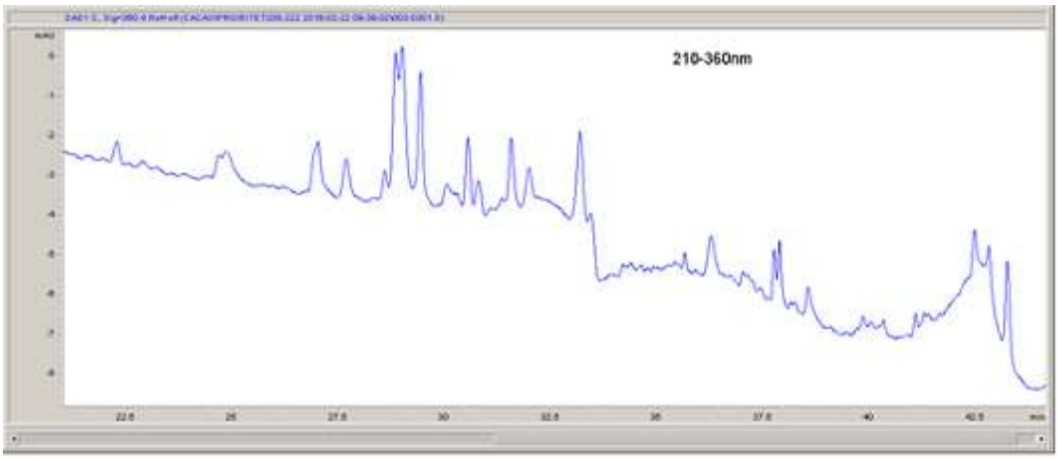


Fig. 2. HPLC chromatogram of acetone extract of *Echium italicum* L.

Plants have been used for centuries to treat infectious diseases and are considered as an important source of new antimicrobial agents (Cowan, 1999). The advantage of compounds of plant origin over synthetic drugs is that the plants have a larger pharmacological complex. Antimicrobial screening of plant extracts and phytochemicals, then, represents a starting point for antimicrobial drug discovery (Amenu, 2014). Table 2 shows the results of antimicrobial activity of acetone extracts of the plants *Anchusa officinalis* and *Echium italicum* against five bacteria. Acetone extracts showed a certain level of antimicrobial activity with MIC values from 3.91 μg/ml to 125 μg/ml, which was a good antimicrobial activity compared to standard antibiotics amracin. The maximum antimicrobial activity was shown by the acetone extract of *E. italicum* against *S. enteritidis* (3.91 μg/ml) and *P. vulgaris* (7.81 μg/ml), and the lowest sensitivity to *L. ivanovii*, *E. aerogenes*, and *S. Typhimurium*. The antimicrobial potential of the plant extract was a consequence of the presence p-Hydroxybenzoic acid and Rosmarinic acid. The hydrophobicity of plants extract and their bioactive compounds contribute in the breaking down of the membrane cells lipid and make them more permeable for the penetration (Sikkema *et al.* 1995). Extensive leakage of critical molecules and ions from bacterial cells will lead to death (Santos and Novales, 2012).

Table 2. Minimum inhibitory concentrations (MIC) of acetone extracts of the plants *Anchusa officinalis* and *Echium italicum* (μg/ml)

plant/bacteria	*Proteus vulgaris*	*Salmonella enteritidis*	*Enterobacter aerogenes*	*Salmonella Typhimurium*	*Listeria ivanovi*
A. officinalis	62.5	62.5	31.25	62.5	31.25
E. italicum	7.81	3.91	125.00	125.00	62.5
Amracin	0.49	0.49	0.97	0.49	0.97

The acetone extract of *A. officinalis* showed the best antimicrobial activity against *E. aerogenes* and *L. ivanovii*, and the lowest sensitivity to *P. vulgaris, S. enteritidis* and *S. Typhimurium*. Manifested antimicrobial activity was a consequence of the presence of pharmacologically active compounds in plant extracts, which is in accordance with the research of Bošković (2017). Plants are characterized by a mixture of different active mechanisms with different pharmacological profiles, thanks to which they can affect several different diseases, unlike synthetic drugs, which are designed to inhibit or stimulate one of the pathways of pharmacological effects (Della Loggia, 2000).

Conclusion

The research confirmed that acetone extracts of the *A. officinalis* and *E. italicum* possess pharmacologically active substances and that they are justifiably used in folk medicine. Also, extracts showed good antimicrobial activity on the tested bacteria, and that it would be useful to further investigate their biological properties.

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ASSESSMENT OF THE INSECTICIDAL AND REPELLENT EFFECT OF *CITRUS BERGAMIA* AND *ALLIUM SATIVUM* ESSENTIAL OILS IN CONTROL OF *TRIBOLIUM CONFUSUM*

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Abstract

Tribolium confusum Du Val. (confused flour beetle) is an important storage pest that is very common in warehouses, silos, attics, or storehouses. This pest reduces the quality, market value, yield of grains, cereals, pasta, spices, nuts and other products by feeding or contaminating products by leaving excrement, shell, and dead bodies. This research was aimed to asses the insecticidal and repellent effect of bergamot and garlic essential oils solution on *T. confusum*. Essential oils were applied in three different concentrations (0.5%, 1%, 2%) in contact and contact-digestive test. For estimating repellent activity, the Y-tube olfactometer was used with two different solution concentrations (1% and 2%). The experiment was performed in four replications at a temperature of 25±1˚C and 45–70% RH, with 10 insects in each. The effects were determined after 24, 48 and 72h of application. The highest efficacy (100%) was accomplished only after 24h with a concentration of 2% for bergamot, in a contact test. In the same test with garlic, the highest value was 60% after 72h of exposure. For the contact-digestive test, the highest efficacy for bergamot was 97.5% after 48h and 52.5% for garlic with the dose rate of 2% after 72h. It was established that bergamot essential oil solutions (1% and 2%) showed repellent activity, as well as garlic in the concentration of 2%, while a concentration of 1% manifested neutral activity. For comparison, it can be concluded that bergamot had a better effect against *T. confusum*, than garlic.

Keyword: *bergamot, garlic, T. confusum, biological protection, essential oil.*

Introduction

Synthetic pesticides can sometimes lose effectiveness due to the development of pest resistance. Some of them are withdrawn because of economic and regulatory reasons (Bernays & Chapman 1994). Also, insecticides can be harmful to the population of beneficial insects like natural enemies and pollinizers. They can reduce the quality of the environment, as well as put the health of producers and consumers at risk (Odukkathil and Vasudevan, 2013; Christofoli et al., 2015). Natural chemicals for plant protection, based on essential oils represent a non-hazardous alternative way of crop protection. This topic is becoming popular and widespread, as a result of the reduction of toxic and harmful chemical compounds (Isman, 2000). Botanical insecticides have novel modes of action against insects that represent a great strategy for preventing resistance development. These compounds degrade rapidly, they usually don't leave harmful residues and can be applied shortly after harvest (Isman, 2000, 2006 & 2008). Bergamot (*Citrus bergamia*) is a plant from the Rutaceae family. The major chemical compounds of the oil are limonene and linalool. Bergamot oil is very precious due to fragrant aromas. The oil is obtained from the peel of ripe fruits by cold squeezing, but this fruit is not edible and is grown only for its oil (Moufida & Marzouk, 2003; Navarra et al., 2015). Garlic (*Allium sativum*) belongs to the Liliaceae family. Garlic essential oil mostly

contains sulfur compounds like diallyl sulfide, diallyl disulfide (Edris & Fadel, 2002; Corzo-Martínez et al., 2007). These oils have already shown good activity against some storage pests like *Sitophilus zeamais*, *Callosobruchus chinensis*, *Tribolium castaneum*, etc. Storage pests can make large quantitative and qualitative losses during the storage process (Rajendran, 2002). *Tribolium confusum* DuVal, the confused flour beetle (Coleoptera: Tenebrionidae), is a worldwide pest of storage and grain products like flour, cereals, rice, pasta, and ed. (Rees, 1995). This insect infests damaged and broken kernels why it is considered to be a secondary pest. Confused flour beetle can be easily found in mills, warehouses, storages, and grain shipments. Infested products have lower weight and quality, followed by the bad smell which is the main reason for marketability reducing (Ojimelulcwe & Adler; Vayias, 2006).
This study was conducted to test the insecticidal and repellent effects of bergamot and garlic essential oil against the *Tribolium confusum*.

Material and Methods

The experimental part (Picture 1) was performed in the summer of 2019 at the Laboratory for Biological Research and Pesticides, Department of Phytomedicine and Environmental Protection, Faculty of Agriculture, University of Novi Sad in Serbia. For the experiment, *T. confusum* adults were used, which were grown in the laboratory on wheat flour in the incubators with a temperature of 25±1°C and 70-80% relative humidity. Essential oils of bergamot and garlic used in this study are commercially purchased. The experiment was set in four replicates in two treatments. For the control treatment, methanol and water were used.
The repellent effect was tested using Y-tube olfactometer. For testing repellent effect two solutions of essential oils were used (1 and 2%). Ten insect adults were placed in the middle tube and the entrance was closed with parafilm to prevent adults exit. Cotton wool was soaked in essential oil solution which was put in one arm (E) of the olfactometer and control cotton wool in the other arm (K). The repellency was determined after 24h and expressed by the pre-referendum index (PI).
The insecticidal effect was assessed by contact and contact-digestive test. Three different concentrations (0.5, 1, and 2%) of essential oil solutions, firstly dissolved in methanol, were used. The bottom of the petri dish was treated with 0.3 ml of essential oil solution and left to dry at room temperature. For the contact digestive test, flour disks were made at the bottom of the petri dish, left to dry, then treated with solutions (0.3 ml). Ten insects were introduced into each petri dish. Petri dished were placed in an air-conditioning chamber with a temperature of 25°C, and relative humidity of 45-70%, with the neon diffuse lighting of 30159 candelas 15h, and 9h of the dark. The effect was assessed after 24, 48, and 72h. Data were processed with Statistica 10 statistical software using a One-way analysis of variance (ANOVA).



Picture 1: Experimental part

Results and Discussion

Bergamot essential oil showed the repellent activity in both concentrations, while the garlic essential oils showed repellent activity in the concentration of 2% and neutral activity in the concentration of 1% (Table 1).

Table 1. Repellent activity

Tea tree essential oil			
T. confusum	**PI 1%**	**PI 2%**	**Repellent activity**
Bergamot	-0.6	-1	Repellent
Garlic	0	-0,4	Neutral/Repellent

-1.00 to -0.10 repellent activity;
-0.10 to +0.10 neutral activity;
+0.10 to +1.00 attractant activity

The results of bergamot and garlic essential oils are presented in Table 2. Both essential oils manifest efficacy on *T. confusum* which is dose-dependent and rise after time. The bergamot essential oil showed much better efficacy on confused flour beetle then garlic oil. The efficiency of 100% with bergamot essential oil was achieved after only 24h in the concentration of 2% in the contact test. Even in the lowest concentration efficacy was pretty high (80%) after 72h. In the contact-digestive test, the efficiency in the concentration of 2% after 72h was also very high with a percentage of 97.5%. In this test, the lowest concentration did not show good results when compared to the contact test. The efficacy was 17.5% at the end of the experiment. The results obtained in the experiment with garlic are not high when compared with bergamot. The highest efficacy of 60% was achieved in the contact test after 72h for the concentration of 2%. The highest efficacy in the contact-digestive test was achieved at the end of the bioassay (52.5%).

Table 2. Effect of essential oils on *T. confusum*

Concentration (%)					**F value**	**P**
Time of exposure	0.50%	1%	2%	Control		
Bergamot contact-digestive						
24h	12.5±0.5b	62.5±1.25bc	92.5±0.5c	0±0a	143.48**	p<0.01
48h	15±0.57b	67.5±1.7bc	97.5±0.5c	0±0a	94.28**	p<0.01
72h	17.5±0.95b	67.5±1.7b	97.5±0.5c	2.5±0.5a	71.61**	p<0.01
Bergamot contact						
24h	77.5±0.5b	95±1b	100±0b	0±0a	275.93**	p<0.01
48h	77.5±0.5b	95±1b	100±0b	0±0a	275.93**	p<0.01
72h	80±1.15b	95±1b	100±0b	2.5±0.5a	127.58**	p<0.01
Garlic contact-digestive						
24h	12.5±0.95b	30±0.81bc	42.5±0.95c	0±0a	22.53**	p<0.01
48h	17.5±0.95b	45±1.29b	52.5±1.25c	0±0a	22.8**	p<0.01
72h	17.5±0.95b	45±1.29bc	52.5±1.25c	2.5±0.5a	17.92**	p<0.01
Garlic contact						
24h	20±1.15b	27.5±0.95b	22.5±0.95b	0±0a	7.36*	p>0.01
48h	57.5±0.95b	55±1.29b	55±0.57b	2.5±0.5a	36.0**	p<0.01
72h	60±1.15b	57.5±0.95b	60±0.81b	2.5±0.5a	40.63**	p<0.01

Values are mean ± standard deviation; * - significant; ** - very significant;

Malacrinò et al., 2016, studied the fumigant and repellent activity of both enantiomers of limonene, a monoterpene usually found in bergamot, against *T. confusum* du Val. R-(+)-limonene is more effective than S-(−)-limonene because it was able to reach 100% of efficacy at a concentration of 85 mg/L air. R-(+)-limonene showed higher repellency compared to the other enantiomer. Campolo et al (2014b) studied the efficacy of five citrus essential oils against *T. confusum*. In their study, they concluded that limonene manifest insecticide effect which matches with our study. Abd El-Aziz and El-Sayed in 2009 reported that garlic essential oil had a low effect to larvae of *T. confusum*, but both showed repellency to adults, which is very similar to the results obtained in this study. Eggs of *T. confusum* are susceptible to garlic as well. Garlic essential oil manifests good effects against numerous storage pests which are well reported (Işikber, 2010; Gözek, 2007; Karci & Işikber, 2007).

Conclusions

According to the achieved results, it can be concluded that bergamot essential oil has an excellent effect on *T. confusum*, while garlic essential oil had a lower effect compared to bergamot. Considering all the advantages of essential oils, they represent a new alternative source of research in the field of crop protection and protection of their products, especially in storage. Still, these are preliminary study which requires more research.

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IDENTIFICATION OF HIGH YIELDING WHEAT GENOTYPES UNDER DIFFERENT ENVIROMENTAL CONDITIONS OF GEORGIA

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Abstract

Eight entries (Attila*2/P8-20HRWYT-5, Tacupeto-F2001/6/CNDO-20HRWYT-225, Kinaci-97, Amsel/TUI//, CUPRA-1/3/CROC1/AE.SQUARROSA(224)//2*OPATA/4 /PANTHEON, KR-11 -9043, KR11-003, KR11-014) with resistant reaction type and low disease severity to rusts selected from different Facultative- and Winter Wheat Nurseries obtained from CIMMYT and ICARDA were tested at field conditions in three sites of Georgia: Meskheti, Javakheti and Shida Kartli. The main agronomic characteristics, like spike length, number of grains in spike, 1000 –kernel weight and average yield of selected genotypes were determined according to the international standards. According to these characteristics all of tested genotypes were superior to the check variety - Bezostaya-1. The data analyses of ecological testing results were done using a two-way ANOVA to estimate the effect of two factors – geographic zone and genotype on the yield and yield components. ANOVA for main characteristics showed that the effects of genotypes were statistically significant at all locations. The effects of geographical zones were not statistically significant.
As results the trials' data analysis the genotype - CUPRA-1/3/CROC1/AE.SQUARROSA (224)//2*OPATA/ 4 /PANTHEON selected from 17FAWWON-SA nursery as improved wheat variety has been submitted for release as a new variety "Lomtagora 143" in Georgia. It is bread wheat variation (var. *lutescens* (Alef.) with 10.5 cm length spikes, 20 good-developed spikelets and 65-70 red, hard grains in each spike; It is characterized by great emergence, strong root system, good tillering and moderate resistant to rusts. 1000- kernel weight is 45 gr, the average yield - 6.5 t/ha. Lomtagora 143 is recommended to produce in Kartli, Meskheti and Javakheti zones.

Keywords: *Wheat variety, Rust resistance, Yield, Georgia.*

Introduction

Wheat (*Triticum aestivum* L.) is the essential food for millions of people and the basis of global food security. Naturally, the development of different types of it's pathogens, including the wheat rusts, which causes the crop harvest's decline in both quantity and quality. Georgia is an area of the permanent spread of rusts because of its climate conditions, diversity of infection reserving plants and existence of alternate host-plants.
Despite its long time existence in Caucasus, (some authors claim that Caucasus is a homeland of yellow rust; Zhukovsky 1965, Stubbs, 1985) stripe rust has created serious problems to the Georgian wheat production only in the near past, which is reflected by the quickened epidemics. During last 20 years 5 stripe rust epidemics (1998, 2000, 2005, 2009 & 2010) have been registered in Central Asia and Caucasus (Bux, 2011; Sharma *et al.*, 2012; Sikharulidze *et al.*, 2015a).
The major and ecologically reliable way to confront this problem is development and release of resistant varieties. In order to protect wheat from rust, cereal rust monitoring global

network has been created and many international organizations (ICARDA, CIMMYT, BGRI, FAO, etc.) prepared numerous programs, like WRDGP, IWWIP). Since 2000 Department of Genetics for Resistance has been involved and actively cooperated with the above mentioned network for years (Bedoshvili *et al.*, 2009).
The research goal was testing the resistance of the introduced breeding material to yellow rust, revealing new sources of resistance, studying their agronomical features, and identification the best- adapted genotypes for production in Georgian conditions.

Material and Methods

Plant material and field trial design. The trials pertaining to the screening of wheat germplasm for their resistance against stripe rust were conducted at the experimental area of Institute of Phytopathology and Biodiversity in 2014. During these investigations, tested international nurseries (17FAWWON-SA, 19FAWWON-SA, 20HRWYT, 17IWWYT-IR, 1st-CACWWYT) obtained from CIMMYT and ICARDA, were sown to observe the stripe rust response in field. Each entry was planted in a 2 m row length, 30 cm apart. Two rows of universal susceptible variety Morocco were planted around the nursery. In addition, a row of Morocco was also planted within the screening material after every 20th entry to enhance inoculum pressure. Evaluation of tested material was carried out under natural and artificial infection. Three replications were made for each of the variety/line.
The wheat yield trials were conducted at three locations: Meskheti (Akhaltsikhe), Javakheti (Akhalkalaki) and Shida Kartli (Mtskheta, Tsilkani) over two growing seasons: 2014-15 and 2015-16 in Georgia. Eight tested entries and local standard variety Bezostaya 1 were planted in all trials in four replications arranged in complete blocks (randomized complete block design) [Kuehl, 2000]. The plot area was the same - 20 m^2 (10m x 2m) - in all trials. Each variety was sown in 10 rows spaced 20 cm apart at a rate 130 seeds per meter. The trial was hand-planted to reduce the experimental error.
Data collection. The Zadoks scale [6] was applied to study phenology and growth stages of the wheat varieties. Observation on response and severity of stripe rust was recorded according to Leogering (1959). The host plant response to the rusts was assessed using the following grades: 'R' to indicate resistance or miniature uredinia; 'MR' to indicate moderate resistance, expressed as small uredinia; "MS' to indicate moderate susceptible, expressed as moderate size uredinia, and "S' to indicate full susceptibility. The disease severity was recorded as % of rust infection on the flag leaf surface area according to the modified Cobb's Scale (Peterson *et al.,* 1948). To analyze of disease progress on breeding materials the coefficient of infection (C.I.), Area Under Disease Progress Curve (AUDPC) was calculated for each entry (Wilcoxon *et al.*, 1975; Stubbs *et al.*, 1986). Disease severity and host response data were combined in a single value called the coefficient of infection (C.I.) were calculated by multiplying the disease severity and a constant value for host response. These values of host response were 0.0, 0.2, 0.4, 0.8 and 1.0 for immune, resistance (R), moderate resistance (MR), moderate susceptible (MS) and susceptible (S), respectively.
Grain yield was measured using a sample of wheat heads that was harvested in 1 m^2 area in each plot. The sample was taken from the middle part of the plot to exclude border effects. The heads were threshed and grain yield was weighed. Average grain yield in grams per plot was calculated and transformed into ton/ha. To measure yield components (productive head/m^2, grain/head and thousand-kernel weight), 50 productive heads from each plot were cut randomly at the ground level prior to harvesting as a sample, placed in paper bags, and then were hand-threshed carefully after drying. Kernels dried once again for 2 days. After drying, they were counted and weighed. Thousand kernel weight (TKW) sampled at random

from all grains harvested from each experimental plot was also measured [Sayre *et al.*, 1997]. Heads were harvested by hand to reduce experimental error.

Data analysis. The data analyses of ecological testing results of above mentioned genotypes were done using a two-way ANOVA to estimate the effect of two factors – Geographic zone and Genotype on the yield and yield components. The statistical analysis included calculation of the experimental error of the field trials and estimation of the least significant differences for separating means of the tested entries. Such an analysis makes possible to determine the range of variation and assess the significance of differences between the trial variants.

Results and Discussion

As shows from table 1, four genotypes (KR11-003, KR11-014, Kinaci-97-17IWWYT-IR-, Tacupeto-F2001/6/CNDO- 20HRWYT - 225,) showed (R) reaction type, very low Coefficient of Infection (0.2) and AUPDC. The rest entries: KR11-9043, Attila*2/P8 -20HRWYT-5, Amsel/TUI//… LG-44, 17FAWWON-SA-CUPRA-1/3/ had (MR) moderate resistance with Coefficient of Infection between 2-8 and AUDPC - from 42 to 220.

Table 1. Characterisics of resistance level of genotypes, selected from International Nurseries

N	Genotype	Reaction	CI	AUPDC
1	Attila*2/P8 -20HRWYT-5	5MR	2	130
2	Kinaci-97- 17IWWYT-IR-9803	R	0.2	22
3	Tacupeto-F2001/6/CNDO, 20HRWYT-225	R	0.2	22
4	Amsel/TUI//… LG-44	10MR	8	220
5	KR-11 -9043	5MR	2.0	42
6	KR11-003	R	0.2	22
7	KR11-9014	R	0.2	22
8	17FAWWON-SA- CUPRA-1/3/CROC1/AE.SQUARROSA (224)// 2*OPATA/ 4 /PANTHEON	10 MR -	8	220

These advanced breeding lines resistant to stripe rust were selected from different International Nurseries for ecological testing to study their yield and yield components. The main agronomic characteristics, like spike length, number of grains in spike, 1000 –kernel weight and average yield of selected genotypes were determined at field conditions in 2015-2016. Nearly all of experimental genotypes showed high levels of grain yield and other agronomic traits. The local check Bezostaya 1 had the lowest grain yield (Table 2).

Table 2. Analysis of variance for grain yield and yield components of wheat genotypes, selected from International Nurseries at three sites in Georgia

#	Sample	Plant height, Cm	Spike length, cm	Number of grains in spike	1000 – kernel weight, (gr)	Average yield, t / ha
1	KR11-9014	95±0.12	9.7±0.16	44.8±0.1	42.5±0.5	5.0±0.14
2	KR-11 -9043	90±0.11	8.6±0.16	48.8±0.11	45.8±0.16	5.4±0.16
3	KR11-003	85±0.09	11.3±2.7	50.6±0.17	44.7±0.2	5.5±0.01
4	Attila*2/P8 -20HRWYT-5	80±0.22	9.8±0.13	49.4±0.9	40.2±0.4	5.0±0.13
5	Kinaci-97, 17IWWYT-IR-9803	90±0.12	10.7±0.08	54.8±0.75	47.1±0.25	5.1±0.19
6	Tacupeto-F2001/6/CNDO, 20HRWYT-225	95±0.12	11.8±0.21	42.2±0.65	45.0±0.18	5.8±0.17
7	Amsel/TUI//… LG-44	85±0.13	9.7±1.2	48.8±0.8	42.8±0.5	5.3±0.1
8	CUPRA-1/3/ CROC1/ AE.SQUARROSA (224) // 2*OPATA/ 4 / PANTHEON(17FAWWON-SA-)	100±0.18	10.5±2.24	65.5±0.75	48.5±0.09	6.5±0.14
9	Bezostaya-1 (check)	90±0.005	7.8±0.11	37.8±0.05	40.5±0,61	4.1±0.12
		LSD_{05} – 1.6	LSD_{05} – 1.6	LSD_{05} – 0.9	LSD_{05} – 1.7	LSD_{05} – 0.4
			LSD_{01} – 2.2	LSD_{01} – 1.3	LSD_{01} – 1.9	LSD_{01} – 0.6

In accordance with the dispersal analysis data for all yield components F actual > F theoretical, that means these values in replications were substantially different. The spike length varied from 8.6 to 11.8 cm and at 95% confidence level the Least Significant Difference (LSD_{05}) was 1.6, and LSD_{01}=2.2 - at 99% confidence level. Number of grains in spike was also varied in genotypes from 42.2 to 65.5 and the Least Significant Difference at 95% confidence level (LSD_{05}) was 0.9, but at 99% confidence level (LSD_{01}) - 1.3. 1000-kernel weight and average yield of genotypes were also different and varied from 40.2-48.5 gr and 4.1-6.2 t/ha, respectively. Accordingly, the Least Significant Difference at 95% confidence level (LSD_{05}) was 1.7 and 0.4 and at 99% confidence level (LSD_{01}) - 1.9 and 0.6, respectively.

ANOVA analysis for main characteristics (spike length, number of grains in spike, 1000 – kernel weight and yield) showed that the effects of genotypes were statistically significant at all locations but the effect of geographical zones was not statistically significant.

As results the trials' data analysis the genotype - CUPRA-1/3/CROC1/AE.SQUARROSA (224)//2*OPATA/ 4 /PANTHEON was superior to the local check and it have been selected as the most improved wheat variety "Lomtagora 143" for release in Georgia.

Wheat variety "Lomtagora 143" is a bread wheat (*Triticum aestivum* L) variation (var. *lutescens* (Alef.), with *red grains,* without awns; After a multiple cross (CUPRA-1/3/CROC1/AE SQUARROSA) it has been selected from 17FAWWON-SA nursery. Variety Lomtagora 143 is characterized by great emergence, strong root system and good tillering. The number of well-developed productive spikes is 570-580 on $1m^2$; The maximum height of

plant is 100cm; It is lodging resistant and easy to trash and is suitable for mechanical harvesting; Spikes are beige, 10.5 cm length with 20 good-developed spikelets and 65-70 red, hard grains in each spike; 1000- kernel weight is 48 gr; The average yield of Lomtagora 143 is 6.5 t/ha. It is recommended to produce in Kartli, Meskheti and Javakheti regions.

Over 20 years new wheat cultivars were introduced into the country via different ways including genotypes developed by international breeding programs. To improve the productivity of winter wheat in the developing countries, the ICARDA and CIMMYT in collaboration with national partners have been working in the framework of the International Winter Wheat Improvement Program. The same field experiments were conducted in Central and West Asia in 2004-2008 to determine the performance of elite winter wheat breeding lines developed by the International Winter Wheat Improvement Program (IWWIP) and to identify superior genotypes that could be valuable for winter wheat improvement or varietal release. Grain yield and agronomic traits of were analyzed. A set of 25 experimental genotypes was identified. The superior genotypes also had acceptable maturity, plant height and 1,000-kernel weight. Among the superior lines, Agri/Nac//Attila and Shark/F4105W2.1 have already been proposed for release in Kyrgyzstan and Georgia, respectively (Sharma et.al., 2010).

As a result of this collaboration several new varieties: Mtskheta 1(https://agris.fao.org/agris-search/search.do?recordID=QY2016800757), Sauli 9, Lomtagora 109, Lomtagora 107, Lomtagora 155, Lomtagora 149, Lomtagora 123, Lomtagora 126 selected from different international nurseries have already been released in Georgia (Sikharulidze et.al., 2015b; http://www.sakpatenti.gov.ge/ media/ page_files/registered_varieties_e.pdf). The cooperation with breeding centers continues to this day.

The given information on wide adaptation of the internationally important winter wheat genotypes demonstrates that the IWWIP program is enriching the germplasm base in the region with superior winter wheat genotypes to the benefit of national and international winter wheat improvement programs.

Conclusions

Thus, some winter wheat lines were identified as high yielding wheat genotypes under different environmental conditions of Georgia, but the superior genotype CUPRA-1/3/CROC1/AE.SQUARROSA (224)//2*OPATA/ 4 /PANTHEON from International Facultative Wheat Nursery (17FAWWON-SA) has been submitted as a new variety "Lomtagora 143" for release in Kartli, Meskheti and Javakheti regions of Georgia.

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PHYTOGEOGRAPHICAL ANALYSIS OF THE VRŠAC VINEYARDS (SERBIA) WEED FLORA

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Abstract

Application of various agrotechnical measures is the key factor defining the composition of the vineyard weed flora. Vineyard weed communities are highly diverse, due to specific environmental characteristics and frequent disturbances of these agroecosystems. Bearing all this in mind and due to a limited number of studies of the vineyard weed flora in Serbia, the aim of this research was to assess the phytogeographical characteristics of the vineyard weed flora in the Vršac vineyards. Field research encompassed the entire vegetation season (March-November) in 2016 and was performed at three localities within the area of the Vršac vineyards. Floristic analysis was done at 60 fixed 1 m^2 plots (48 between-row and 12 in-row plots). Phytogeographical analysis has determined that 97 weed species which were recorded belong to eight areal types. Weed species predominately belonged to the Eurasian areal type, with 49 recorded species (51%). Results have shown the presence of 14 species of cosmopolitan distribution, while the Pontic-Mediterranean areal type was represented by 10 weed species (10%). This transitional areal type was represented by four areal groups, of which the Pontic-CentralAsian-Submediterranean group was the most numerous. Of the plants typical for the Pannonian region of Serbia, i.e. the steppe species, belonging to the Pontic-SouthSiberian areal type, only two weed species (*Asperugo procumbens* and *Senecio vernalis*) were recorded. Allochthonous species (adventive areal type) were represented by eight weed species, with species originating from North America (*Amaranthus retroflexus*, *Erigeron annuus*, *E. canadensis*, *Panicum capillare* and *Xanthium strumarium* subsp. *italicum*) being dominant.

Keywords: *areal type; biogeography; floral element; Vojvodina province; Vršac vineyards.*

Introduction

The tradition of winegrowing has for centuries been one of the main agricultural activities on the southern slopes of the Vršac mountains (South Banat region, Autonomous Province of Vojvodina, Serbia; PSUZŽS, 2018). Weeds compete with grapevines, thus affecting the plant vigor, final yield, and the quality of wine itself (Dujmović Purgar and Hulina, 2004; Ingels et al., 2005). Diversity of vineyard weed communities is the result of specific environmental conditions and frequent disturbances of these agroecosystems (Gago et al., 2007). These weed communities are highly dependent on the human factor (Kovačević et al., 2015), with various agrotechnical measures affecting their composition (Gago et al., 2007). Both in-row and between-row weed control is of vital importance for maintaining the vineyards weed-free and reducing the weed seedbank (Fredrikson et al., 2011), equally affecting the weed flora. Bearing all this in mind and due to a limited number of studies of the vineyard weed flora in Serbia, the aim of this research was to assess the phytogeographical characteristics of the vineyard weed flora in the Vršac vineyards.

Material and Methods

Field research was done once per month in the March-November period of 2016, with the aim to encompass all vegetation aspects during the vegetation season. The floristic analysis was performed at three localities within the Vršac vineyards area: Magareći breg, Izlaz-Šeribl and Kozluk-Majdan, at 60 fixed 1 m^2 plots - 48 between-row and 12 in-row plots, distributed evenly between the field sites. The recorded weed species were identified in the field or collected and subsequently identified, using the standard identification keys: Josifović (1970-1977), Javorka and Csapody (1975) and Sarić (1992). Nomenclature of the weed species follows the Euro+Med PlantBase (Euro+Med, 2006-2020).
Weed species classification based on floristic elements into appropriate phytogeographical units (areal types and areal groups) was done according to Gajić (1980) and the chorological classification of Meusel et al. (1965, 1978), with necessary regional modifications of the classification as defined by Stevanović (1992) for the territory of Serbia. The spectrum of the represented areal types and areal groups is given as a percentage of the total number of recorded species. An index of floristic originality (OR) was computed following Malyshev (1991): OR = (S –Ŝ)/S, where S and Ŝ are the actual and expected species numbers. The expected species number is calculated by following the method of least squares (Malyshev, 1969 *in* Malyshev, 1991): $\hat{S} = 314.1 + 0.004538 * G^2$, where G is the observed number of genera.

Results and Discussion

Field research in vineyards has determined the presence of 97 weed species, belonging to 26 families (Anđelković et al., *in press*). Subsequent phytogeographical analysis has determined that the recorded species belong to eight areal types (Figure 1), divided into 17 different areal groups (Table 1). The number of recorded floral elements is similar to those recorded by Kovačević et al. (2008) for the vineyards in the Herzegovina vineyard region.

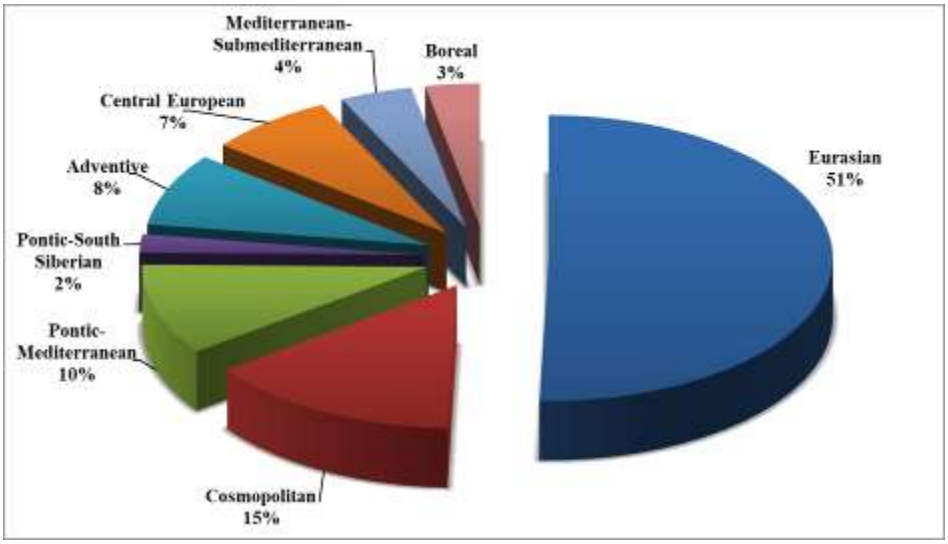


Figure 1. Phytogeographical spectrum of the vineyard weed flora in the Vršac vineyards

The results (Figure 1) have shown that the recorded weed species predominately belong to the Eurasian areal type, with 49 recorded species (51%). Such an abundance of Eurasian species was expected, given that this floristic chorion had the strongest impact on the floristic origins of the flora in the study area (Gavrilović, 2016). Additionally, the dominance of Eurasian

species is also easily explained, knowing their wide geographical distribution and broad ecological valence (Jušković et al., 2010; Tmušić et al., 2019).

Given that the same ecological characteristicsare also true for cosmopolitan species, it is not surprising that this areal type is the second most represented group of species, with 14 weed species (Figure 1). A similar prevalence of Eurasian and cosmopolitan species has also been recorded in the vineyards of Bosnia and Herzegovina (Kovačević et al., 2008; 2015) and the regional potato weed flora (Stešević and Jovović, 2005; Nikolić et al., 2013; Mehmeti et al., 2019). In addition to their ecological characteristics, arelatively high incidence of cosmopolitan species (15%, Figure 1), can be explained by the fact that plant species belonging to this group are common agricultural weeds, and therefore understandably more abundant in agroecosystems (Kovačević et al., 2008; 2015) and ruderal areas (Jakovljević and Jovanović, 2005), when compared to natural habitats in Serbia (e.g. Petrović et al., 2007; Jušković et al., 2010), where they are among the least represented floral elements.

The presence of transitional areal types, i.e. Pontic-Mediterranean and Pontic-SouthSiberian is the result of varying influences affecting the biogeography of the Pannonian part of Serbia (Gavrilović, 2016). Weed species of the Pontic-Mediterranean areal type represent the third most represented group, with 10 species (10%; Figure 1). This transitional areal type is represented by four areal groups: Subpontic-Submediterranean, Pontic-CentralAsian-Submediterranean, Pontic-EasternSubmediterranean and Pontic-Submediterranean areal group, of which the Pontic-CentralAsian-Submediterranean group was the most numerous, with 40% of species within this areal type (Table 1).

Of the plants belonging to the Pontic-South Siberian areal type, i.e. steppe-like species, only two weed species (*Asperugo procumbens* and *Senecio vernalis*) were recorded. Although a higher presence of typical steppe vegetation species was expected, given the geographical position of the study area, the presence of only two such species is a result of specific agroecological conditions and weed control measures which have been applied in the Vršac vineyards for a long period of time. A similarly small presence of this areal type was also recorded in the ruderal flora by Jakovljević and Jovanović (2005), which points to the fact that this floristic element, although generally expected in this geographic area, is much more prevalent in those habitats where the environmental conditions are favorable for the development of such xerothermic, steppe-like vegetation (Ranđelović et al., 2007).

The calculated index of floristic originality is -2.49, with the expected number of species being 338. Such a negative value of OR indicates a strong presence of allochtonous plant species in the study area (Malyshev, 1991). Consequently, the presence of eight allochthonous species, belonging to the adventive areal type is not surprising. Species originating from North America (*Amaranthus retroflexus, Erigeron annuus, E. canadensis, Panicum capillare* and *Xanthium strumarium* subsp. *italicum*) are dominant (Table 1) within this floral element. A similar number of adventive species was also characteristic for weedy-ruderal community of invasive *Asclepias syriaca* (ass. *Asclepiadetum syriacae* Láníková in Chytrý 2009) documented by Popov et al. (2016), highlighting that the high incidence of allochtonous plant species is not uncommon in weedy vegetation. A strong presence of both adventive and cosmopolitan areal types (Figure 1) is indicative of frequent disturbances and strong anthropogenic pressures, characterizing both ruderal (Jakovljević and Jovanović, 2005; Gavrilović, 2016) and agroecosystems, such as vineyards.

A similarly low proportion of floral elements belonging to the Pontic-SouthSiberian, CentralEuropean and Boreal areal types has been recorded in the vineyards of the Herzegovina region (Kovačević et al., 2008). Our results have shown that the CentralEuropean areal type was represented by seven weed species in the weed flora of the Vršac vineyards, most of them (85%) belonging to the SubcentralEuropean areal group (Table 1). Given that these species are predominantly characteristic for the mesophilous deciduous

forests of the temperate zone, a relatively small presence of CentralEuropean species in the vineyard weed flora is not surprising, knowing the ecological conditions prevailing in this perennial agroecosystem. Finally, three species of the boreal areal type were documented in the study area: *Poa pratensis*, *Rubus idaeus* and *Rumex acetosella*, making 3% of the total number of recorded species (Figure 1). As vineyards positioned on the southern slopes of the Vršac mountains are not favorable habitats for the development of plants characteristic for colder and more humid northern regions (Jušković et al., 2010), their poor representation in the total vineyard flora was to be expected.

Table 1. Phytogeographical spectrum of the vineyard weed flora in the Vršac vineyards

Areal type	**number of species**	**%**	**Areal group**	**number of species**
Eurasian	49	51	Eurasian	28
			Sub-Eurasian	21
Cosmopolitan	14	14		
Pontic-Mediterranean	10	10	Subpontic-Submediterranean	3
			Pontic-Central Asian-Submediterranean	4
			Pontic-EasternSubmediterranean	1
			Pontic-Submediterranean	2
Pontic-SouthSiberian	2	2	Subpontic-Central Asian	1
			Sub-south Siberian	1
Adventive	8	8	North America	5
			Asia	2
			North and Central America	1
Central European	7	7	Central European	1
			Subcentral European	6
Mediterranean-Submediterranean	4	4	Mediterranean	1
			Sub-Mediterranean	3
Boreal	3	3	Circumpolar	1
			Subcircumpolar	2

Conclusions

Phytogeographical analysis has shown that weed flora of the Vršac vineyards belongs to eight areal types, with 17 different floristic elements identified. Eurasian species are the most dominant species group (with 51%), followed by cosmopolitan species (15%). The obtained phytogeographical spectrum dominated by species of wide distribution and high ecological plasticity is to be expected in a vineyard ecosystem often disturbed by anthropogenic activities. Of the remaining areal types represented to a lesser degree, the adventive areal type (8%) is of importance, owing to the invasive potential of non-native plant species.

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CONTROL OF *MYZUS PERSICAE* SULZER IN PAPRIKA IN GREENHOUSE

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Abstract

In this study, the possibility of the use of imidacloprid and thiamethoxam in the control of aphids in paprika, under greenhouse conditions, was evaluated. Significant pests, such as *Myzus persicae* Sulzer (Aphididae) causes decreased growth, shriveling of the leaves and transmitting plant viruses. The trials were conducted in 2018 by standard OEPP/EPPO methods at locality Bačko Gradište (Vojvodina) in paprika crop (hybrid Ariadni) in the greenhouse. The insecticides based on imidacloprid (200 g a.i./l, SL) were applied at an amount of 0.6 l/ha and thiamethoxam (250 g a.i./kg, WG) at an amount of 0.18 kg/ha. Insecticides were foliar applied, using a backpack sprayer with water consumption of 400 l/ha. Efficacy (E%) of the insecticides was determined according to Henderson & Tilton, while the significance of differences was evaluated by LSD test (5%). Before the treatment, the average number of aphids ranged from 40.1 to 51.6 per leaf. The first day after the application of the insecticides, the number of *M. persicae* was significantly lower in comparison to the control, and the efficacy was 92.3-95.3%, while eight days after the treatment efficacy was 99- 99.4%. Fifteen days after the application, the efficacy was still high in the range of 99.4-99.8%. On the control variant during the trials, a large increase in the population of aphids was detected, which shows us the importance of its timely control, to be started by the formation of a colony. The population of *M. persicae* in the paprika crop in the greenhouses at Bačko Gradište showed high susceptibility to insecticides from the neonicotinoid group.

Key words: *paprika, greenhouse, Myzus persicae, insecticides*

Introduction

Paprika has great economic importance since it is grown in large areas. In the food industry, it is a very important raw material for canning products, as well as in the spice industry, freezing and drying. It is one of the most important vegetable plants, primarily due to its highly nutritious and biological value (Marković and Vračar, 1998). According to the areas on which it is grown and the yield achieved by paprika, it is one of the most important vegetable plants in Serbia. It is one of the most cultivated and one of the most profitable crops in the greenhouse due to extremely long vegetation, which brings about high yield (Lazić et al., 1998). Growing paprika in a greenhouse allowed the possibility for cultivation in the areas where paprika cannot be grown in the open field. Numerous harmful insects, including Aphids, can endanger the successful production of this crop. Plant aphids (Hemiptera: Aphididae) are an economically important group of insects, especially in areas with a continental climate (Petrović et al., 2017). *Myzus persicae* is widespread, especially in the areas with numerous peach orchards which are its winter host. Both adults and larvae cause weakening of plants by sucking sap. Besides this they act as vectors for plant viruses.The green peach aphid transmits over 180 types of viruses (Kereši et al., 2016). Today, intensive crop production with rational yield is unimaginable without the use of chemicals. Considering environmental and human health, as well as financial aspect, the use of plant protection

products should be reduced to a minimum. They should be used in a controlled manner, to suppress one or a group of pests, and only when other possibilities have been exhausted and in the case of economically significant damage. Chemical control of aphids should be conducted with the first appearance of the colonies. The critical number is 20% of infected plants (Kereši, 2010).
The aim of this study was to determine the degree of paprika protection in greenhouse conditions against aphids (Aphididae), using preparations based on imidacloprid and thiamethoxam.

Material and methods

The trials were conducted in 2018 by standard OEPP methods for the design and analysis of efficacy, as well the phytotoxicity assessment (Anonymus, 2004; Anonymus, 2012; Anonymus, 2014) at locality Bačko Gradište (Vojvodina) in paprika crop (hybrid Ariadni) in the greenhouse. The pesticide preparations based on imidacloprid (200 g a.i./l, SL) were applied at an amount of 0.6 l/ha and thiamethoxam (250 g a.i./kg, WG) at an amount of 0.18 kg/ha. Pesticides were foliary applied, using a backpack sprayer with water consumption of 400 L/ha when paprika were in the stage BBCH 71.



Photo 1-2. Paprika crop in greenhouse (B. Gradište); *M. persicae* on the paprika leaf (Foto: original, 2018)

Experiment in Bačko Gradište was set on august 2018. The experiment was carried in four replications, by randomized complete block design. Four assessments were made based on the number of aphids on 10 previously marked leaves on five plants per replication: 1) before the treatment, 2) one day after the treatment, 3) eight days after the treatment, 4) 15 days after the treatment. The efficiency of the insecticides was calculated by Henderson & Tilton (Wentzel, 1963) and the significance of differences was determined by ANOVA for a confidence interval of 95%.

Results and discussion

The results of testing the efficacy of insecticides based on in greenhouse production conditions for controlling *M. persicae* are shown in Tables 1-2. Based on the abundance estimate just before the trial was set up (Table 1), the average abundance of *M. persicae* by variants ranged from 401.0 to 516.5. One day after application of imidacloprid and thiamethoxam, the abundances of *M. persicae* was significantly lower than the control. The

efficacy of the tested preparations ranged from 92.3 to 95.3% and they are at the same level of significance.

Table 1. Average number of green peach aphids (*M. persicae*) and insecticide efficacy (B. Gradište, 2018.)

Insecticides (l,kg/ha)	before treatment		one day after the treatment		
	$\bar{x}$	±Sd	$\bar{x}$	±Sd	E%
imidacloprid (0.6)	**499.3 a**	85.51	**48.2 b**	11.98	**92.3**
thiamethoxam (0.18)	**401.0 a**	67.28	**29.3 b**	3.86	**95.3**
control	**516.5 a**	57.46	**626.7 a**	73.5	/
LSD 5%	**147.4**		**89.3**		

$\bar{x}$ – average number; ±Sd - standard deviation; E %- efficacy; F=3.076, p=0.096; =248.7, p<0.01

The number of aphids, eight days after the treatment, was at a significantly lower level compared to the control. The efficacy of the tested preparations was 99-99.4%. The number of green peach aphids 15 days from the application of the insecticides is significantly lower than the control. The preparation efficiency was still high and ranged from 99.4 to -99.8% (Table 2).

Table 2. Average number of green peach aphids (*M. persicae*) and insecticide efficacy (B. Gradište, 2018.)

Insecticides (l,kg/ha)	eight days after the treatment			15 days after the treatment		
	$\bar{x}$	±Sd	E %	$\bar{x}$	±Sd	E%
imidacloprid (0.6)	**9.2 b**	5.12	**99.0**	**13.0 b**	3.74	**99.4**
thiamethoxam (0.18)	**4.75 b**	2.22	**99.4**	**4.5 b**	2.38	**99.8**
control	**934.7 a**	56.9	/	**2899.5a**	673.0	/
LSD 5%	**68.5**			**805.9**		

$\bar{x}$ – average number; ±Sd - standard deviation; E %- efficacy; F=1053.0, p<0.01; F=73.8, p<0.01

Jeschke and Nauen (2008) state that compounds from the neonicotinoid group are an important class of insecticides in integral agriculture and in the anti-resistance strategy in the control of insect pests. Data from Nauen and Denholm (2005) indicate the existence of cross-resistance of some populations of *M. persicae* and *Bemisia tabaci* to acetamiprid and imidacloprid. In greenhouses in Israel, *B. tabaci* developed resistance to acetamiprid 5-10 times, after three years of its use for controlling this pest (Horowitz et al., 1999). The studies of Margaritopoulos et al. (2006), in Greece on tobacco showed different levels of resistance of several insect species to imidacloprid, including *M. persicae*. This development of resistance have been attributed to the resistance to the target site of action or by improved detoxification. Thiamethoxam has been used as a main insecticide to control *B. tabaci* for several years in China without the presence of resistant populations. To assess the risk of resistance, field populations were collected after which they were exposed to thiamethoxam in laboratory conditions. After the selection of 36 generations, individuals with a resistance of 60 times were successfully identified (Feng et al., 2009).

In recent years, neonicotinoids have proven to be insecticides to which insects are relatively difficult developing resistance, especially when considering family like *Aphididae* with

representatives such as *M. persicae* and *Phorodon humuli*. Although the susceptibility of *M. persicae* can vary up to 20 times among populations, this does not significantly reduce the efficacy of neonicotinoids (Nauen and Denholm, 2005). In our trials the population of *M. persicae* in the paprika crop at the locality of Bačko Gradište, in greenhouse production, showed high sensitivity to insecticides from the neonicotinoids group.

After the European Union limited the use of neonicotinoids due to their harmful effects on pollinators, primarily bees, considering numerous evidence of losses of bee colonies as well, in 2019 their ban was introduced in all crops and orchards in the open field. Their use is allowed only in greenhouses, while the use of the active substance thiamethoxam is completely prohibited.

Conclusions

Based on the performed tests and the obtained results in suppressing aphids (Aphididae) for the paprika crop in the greenhouse, the following conclusions can be drawn:

- The effectiveness of insecticides based on imidacloprid and thiamethoxam for controlling aphids (*Aphididae*) was high (92.3 - 99.8%) in all evaluations.
- The control variant showed a large increase in the population of aphids, which indicates the importance of their timely control, i.e. at the beginning of the colonies' formation.
- The population of green peach aphid (*M. persicae*) in the paprika crop at the locality of Bačko Gradište, in greenhouse production, showed high sensitivity to insecticides from the neonicotinoids group.

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DISSIPATION RATE OF BOSCALID AND PYRACLOSTROBIN FUNGICIDE IN STRAWBERRY AND RASPBERRY FRUITS

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Abstract

This study was conducted in order to obtain the half-life of boscalid and pyraclostrobin fungicides in strawberry and raspberry fruits and to evaluate pre-harvest interval (PHI). The field trials were carried out in 2019 at two sites, located in Republic of Serbia. Strawberries and raspberries were sprayed with the commercial formulation (267 g a.i./kg boscalid and 67 g a.i./kg pyraclostrobin) in an application rate of 1.5 kg/ha. The samples of mature fruits were randomly collected after drying deposit and each day for one week. The extraction was performed using a QuEChERS based method, while for the simultaneous determination of boscalid and pyraclostrobin residues in strawberry and raspberry fruits HPLC/DAD was used. The method was developed and validated in accordance with SANTE/11813/2017 and applied on the real samples. The highest content of boscalid and pyraclostrobin residues in strawberry (10.23 mg/kg and 4.23 mg/kg) and raspberry (10.6 mg/kg and 7.63 mg/kg) fruits were immediately after drying the deposit. The residues of boscalid and pyraclostrobin in strawberry fruits, at the MRL level of 6 mg/kg and 1.5 mg/kg, respectively, were reached between 3^{rd}-4^{th} and the 4^{th}-5^{th} day after treatment and the residues in raspberry fruits, reached the MRL level (10 mg/kg for boscalid and 3 mg/kg for pyraclostrobin) at first and fourth day after the application. The results suggested that the boscalid and pyraclostrobin dissipation curves followed the first-order kinetic and their half-lives were 3.89 and 2.40 days in strawberries, and 4.1 and 2.77 days in raspberries. At the end of PHI (seven days), residues of both fungicides in strawberry and raspberry fruits were far below the MRLs.

Keywords: *boscalid, pyraclostrobin, strawberry, raspberry, dissipation.*

Introduction

The growing of berry fruits requires intensive protection against pests and diseases. Strawberries and raspberries are particularly susceptible to diseases mostly caused by various fungal infections (Sadło et al., 2018). The main disease of berry fruits is grey mold (*Botrytis cinerea* Pers.). The presence of *B. cinerea* may affect the taste and appearance of fruit (Mitchell et al. 2010), and cause problems during storage and transportation (O'Neill et al. 2012). This pathogenic fungus may occur several times during the growing season, which requires the intensive application of plant protection products (PPPs) for its suppression. For the control of *B. cinerea* in strawberry and raspberry application of PPPs based on boscalid and pyraclostrobin has shown high efficiency. They are applied repeatedly, starting from the flowering period, up to 7 days before harvest, at intervals of 7-10 days. This product is also used against anthracnose (*Colletotrichum fragariae*), leaf scorch (*Diplocarpon earliana*), common leaf spot (*Mycosphaerella fragariae*) and Raspberry spur blight (*Didymella applanata*).

However, PPPs leave residues that can be accumulated at higher levels than prescribed maximum residue levels (MRLs) which makes them potentially harmful to the consumers' health. Maximum residue levels of boscalid and pyraclostrobin fungicides in strawberry fruits

in the EU, as well in the Republic of Serbia, are 6 mg/kg and 1.5 mg/kg, while in raspberry fruits are 10 mg/kg and 3 mg/kg, respectively. One of the most important parameters in assessing the fate of pesticide residues (Li et al., 2016) is the dissipation rate and it can be used to estimate the required time for bringing residues down below MRLs (Ambrus and Lantos, 2002). Also, the dissipation of pesticides after application may be affected by different factors such as plant species, pesticide chemical structure, type of formulation, application method, climate, and photo degradation (Lazic et al., 2018).
In this study, a field experiment was performed in order to evaluate residue levels and dissipation dynamics of boscalid and pyraclostrobin in strawberry and raspberry fruits, under Serbian agroecological conditions.

Material and Methods

The field trials were carried out in 2019 at two sites (Gospodjinci and Trnava) located in the Republic of Serbia. Plant protection product based on boscalid and pyraclostrobin fungicides is used in the control of *Botrytis cinerea*, fungi causing gray mold, in berry fruits. Strawberries (variety Joly) and raspberries (variety Polana) were sprayed with the commercial formulation (267 g a.i./kg boscalid and 67 g a.i./kg pyraclostrobin) in the application rate of 1.5 kg/ha, according to the manufacturer's recommendation, when fruits were in the BBCH 65-87 scale.



Figure 1. Extraction and clean-up procedure (photo original)

For the fungicide residues analysis, the samples of mature fruits were randomly collected after drying deposit, and each day for one week. Untreated samples were collected as well and used for method validation.

The extraction of boscalid and pyraclostrobin was performed using a QuEChERS based method (Anastasiades *et al.*, 2003) (Figure 1). For the simultaneous determination of boscalid and pyraclostrobin residues in strawberry and raspberry fruits, HPLC/DAD was used. The mobile phase was deionized water acidified with H_3PO_4 (pH 2.8) and acetonitrile (25/75, v/v), flow rate 0.55 ml/min, column temperature 25 °C, injected volume 20 µl, while 230 nm wavelength was applied. Under these conditions, the retention time of boscalid and pyraclostrobin was 1.556 minute and 2.333 minute. The method was developed and validated in accordance with SANTE/11813/2017 and applied to the real samples.

Results and Discussion

Method validation. Validation of the method was evaluated through the linearity, precision, recovery, limit of detection (LOD) and quantification (LOQ) as well as, the matrix effect (Table 1). The results obtained for the parameters completely fulfilled SANTE/11813/2017.

Table 1. Validation parameters

Fungicide	LOD (mg/kg)	LOQ (mg/kg)	R^2	Precision (%)	Recovery (%)	Matrix effect (%)
Boscalid	0.05	0.2	0.999	0.47	76-92	89.4
Pyraclostrobin	0.01	0.04	0.977	6.90	74-89	87.9

Fungicides residues in strawberry fruits. Results obtained for the analysis of boscalid and pyraclostrobin residues in strawberry fruits are presented in Table 2. The highest content of boscalid and pyraclostrobin residues in strawberry (10.23 mg/kg and 4.23 mg/kg) fruits were immediately after drying the deposit. During the following seven days, the content of boscalid and pyraclostrobin continuously decreased. Maximum residue levels (MRLs) of boscalid and pyraclostrobin are 6 mg/kg and 1.5 mg/kg, respectively.

Table 2. Dissipation of boscalid and pyraclostrobin in strawberry fruits

Days after the application	Boscalid			Pyraclostrobin		
	Residue (mg/kg)	Persistence (%)	Loss (%)	Residue (mg/kg)	Persistence (%)	Loss (%)
0.	10.23	100.00	0.00	4.23	41.35	58.65
1.	9.23	90.22	9.78	3.16	30.89	69.11
2.	7.36	71.95	28.05	2.23	21.80	78.20
3.	6.23	60.90	39.10	2.01	19.65	80.35
4.	5.32	52.00	48.00	1.54	15.05	84.95
5.	4.15	40.57	59.43	1.23	12.02	87.98
6.	3.16	30.89	69.11	0.93	9.09	90.91
7.	3.01	29.42	70.58	0.39	3.81	96.19

The residues of boscalid and pyraclostrobin in strawberry fruits, at the MRL, were reached between 3^{rd}-4^{th} and the 4^{th}-5^{th} day after the treatment. Seven days after the application, the content of boscalid (3.01 mg/kg) and pyraclostrobin (0.39 mg/kg) in strawberry fruits was far below MRL.

Using the obtained results, the half-lives (DT_{50}) of the analyzed fungicides were determined. The dissipation kinetics of the boscalid and pyraclostrobin in strawberries and raspberries were determined by plotting residue content against time. The half-life of fungicides in

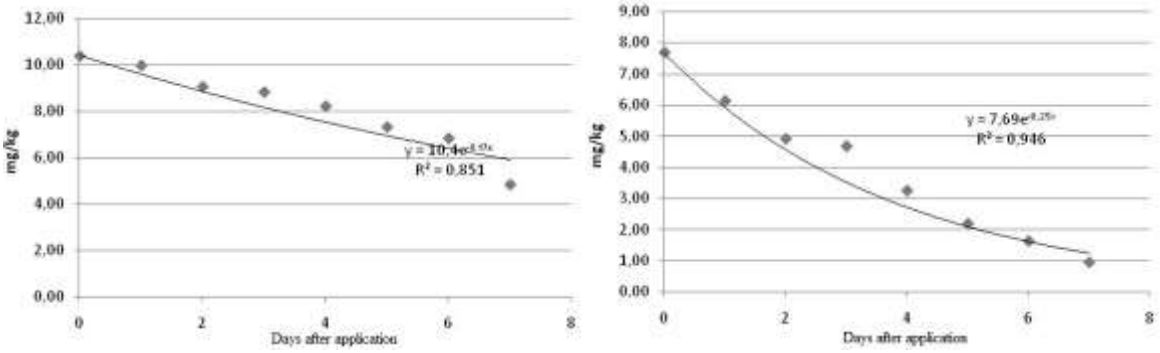
different matrices was calculated using the equation $C_t=C_0e\text{-}kt$ where C_t represents the concentration of the pesticide residue at time t, C_0 represents the initial concentration and k is the rate constant per day. DT_{50} were determined from the k value ($DT_{50}=ln2/k$) (Gupta and Shanker, 2008).

Table 3. Half-life (DT_{50}) of boscalid and pyraclostrobin in strawberry fruits

Fungicide	Regression equation	Constant	R^2	DT_{50} (day)
Boscalid	$y= 10.23e^{-0.178x}$	0.178	0.9846	3.89
Pyraclostrobin	$y= 4.23e^{0.284x}$	0.284	0.934	2.40

The regression coefficient of boscalid (0.984) and pyraclostrobin (0.934) indicates a continuous and gradual decrease of both compounds. Boscalid and pyraclostrobin half-lives in strawberries fruits obtained in this experiment, after application in the recommended amount, were 3.89 and 2.40 days.

Fungicides residues in strawberry fruits. Dissipation dynamics of the above-mentioned fungicides were evaluated in raspberry fruits, as well. Analysis of boscalid and pyraclostrobin residues in raspberry fruits showed the highest concentration (10.4 mg/kg and 7.63 mg/kg, respectively) one hour after the application (Graph. 1 and 2). After two days, the concentrations of boscalid were 9.63 mg/kg, lower than prescribed MRL of 10 mg/kg. The MRL level of pyraclostrobin in raspberry fruits (3 mg/kg) was achieved four days after the application of PPPs (2.21 mg/kg).



Graph 1 and 2. Dissipation curve of boscalid and pyraclostrobin in raspberry fruits

Table 4. Half-life (DT_{50}) of boscalid and pyraclostrobin in raspberry fruits

Fungicide	Regression equation	Constant	R^2	DT_{50} (day)
Boscalid	$y= 10.4e^{-0.17x}$	0.17	0.851	4.10
Pyraclostrobin	$y= 7.69e^{0.25x}$	0.25	0.946	2.77

Using these results, the half-life of boscalid and pyraclostrobin in raspberry fruit was calculated (Table 4). The first-order kinetic equation determination coefficients (R2) were 0.851 and 0.946. Boscalid and pyraclostrobin residues found in the analyzed raspberry samples indicated half-lives of 4.1 days and 2.77 days, respectively.

In the study conducted by Chen and Zhang (2010) boscalid dissipation pattern followed the first-order kinetics with the half-lives of 4.9 and 6.4 days in strawberries. The boscalid residues in strawberries were below the EU MRL after three days of application. During 2015 and 2016 dissipation dynamics of the PPPs based on boscalid and pyraclostrobin were

analyzed at two localities in China (Yang et al., 2018). The residues of fungicides were simultaneously determined in strawberry and soil by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). The samples were extracted by acetonitrile, purified by N-propyl ethylenediamine (PSA). Under these conditions, the half-lives for the dissipation of boscalid and pyraclostrobin were 4.8-6.0 days and 5.1-11 days, respectively, in strawberry, and 3.4-10.0 days and 3.4-6.0 days, respectively, in soil.
The dissipation study of boscalid in raspberry fruits was conducted in Poland, during 2013–2014. The field trials were conducted on a plantation of the Laszka variety dessert raspberry. The half-life of boscalid in ripe raspberry fruit was slightly below 7 days, while the mean pyraclostrobin residue levels dropped by half within the first 6 days of fruits picking (Sadło et al., 2018).

Conclusion

This study suggests that boscalid and pyraclostrobin could be safely used in strawberries and raspberries in the recommended dosage in the Serbian agroecological conditions. The half-lives of boscalid in strawberry and raspberry fruits were 3.89 days and 4.10 days, while the half-lives of pyraclostrobin in strawberry and raspberry fruits were 2.40 and 2.77 days, respectively. At the end of PHI of seven days, residues were far below the MRLs.
It is important to note that boscalid and pyraclostrobin residues were analyzed after a single application. It would be of great importance to conduct research in order to determine the residues of these fungicides at the end of the pre-harvest interval, after several applications.

Acknowledgement

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EFFICACY OF FUNGICIDES AND FERTILIZERS IN THE CONTROL OF *XANTAMONAS EUVESICATORIA* IN PEPPER

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Abstract

Bacterial spotty of peppers formerly referred to as *X.campestris* under our conditions in pepper growing areas such as Ruski Krstur, Sombor occurs regularly in field crops and is the most significant factor limiting the yields of peppers especially on some varieties. The molecular method of the various strains revealed that they all belong to the species *Xanthomonas euvesicatoria*. The first symptoms occur in the field in early July, after a rainy season, on the lower older leaves in the form of tiny dark green and moist spots. In conditions of humidity and higher temperatures, diseased tissue turns brown. The spotting occurs and large necrotic spots are formed. Lower leaves with the higher and stronger intensity of bacteriosis, yellow which can lead to decrease. At higher intensities, *Xanthomonas* infects the fruit as well, giving symptoms such as scabies. *X.euvesicatoria* is transmitted year by year through infected diseased dead tissue and through infected semen. Symptoms can occur as early as seedlings and subsequently appear more widely in the field. On the plant, bacteriosis spreads with raindrops or, if it is poured over the plants, with water. Bacteria carried by the wind are transferred from plant to plant. *X. euvesicatoria* have cilia, swim in the water and, upon reaching the stoma, infect the plant. Most varieties of peppers are sensitive to *X. euvesicatoria*. However, in field conditions it occurs regularly in areas where peppers are grown on large areas. Copper-based preparations are used to protect the peppers, which must be used frequently and since it acts only in contact, the effectiveness is relatively limited. In order to test the effectiveness of the registered preparations in the trial, we also used newer preparations in pepper protection. The experiment was performed in the PSS Sombor field experiment on the Bagoly F1 (Hungary) variety, which has elongated fruits, extremely thick meat with 3-4 chambers. The experiment had 4 variants in 3 repetitions. To evaluate the effectiveness, we used the number of spots on the experimental surface of 10 plants in three replicates. The harvest was done twice on 5.08 and 31.08.2019. We evaluated the number of healthy fruits and the total yield.

Keywords: *Xanthomonas euvesicatoria, pepper, fungicides, fertilizers, protection*

Introduction

Bacterial spot of peppers formerly referred to as *X.campestris* in our conditions in areas of pepper cultivation such as RuskiKrstur, Sombor regularly occurs in field conditions and the most important factor that limits the yields of peppers, especially in some varieties.In studying the diversity of the population of Xanthomonas spp.pathogen on pepper Ignjatov (2013) found by applying the molecular method different strains but that they all belong to the species *X.euvesicatoria*. The first symptoms appear in the field at the beginning of July, after the rainy period, on the lower older leaves in the form of small dark green and moist spots.In conditions of humidity and higher temperatures, the diseased tissue turned brown.Spots merge and large necrotic spots form.Lower leaves with a higher and stronger intensity of bacteriosis,

which can lead to decline.At higher intensities, Xanthomonas also infects fruits, resulting in scab-like symptoms on the fruit and losing commercial value.



Photo 1. Necrotic spots on peppers caused by *X.euvesicatoria*

X.euvesicatoria is transmitted from year to year through infected diseased dead tissue and through infected seeds. Symptoms can appear already on the seedling and later appear more massively in the field. On plants, bacteriosis is spread by raindrops or, if watered over plants, with water. Bacteria carried by the wind are transmitted from plant to plant. *X euvesicatoria* have cilia, swim in the water and infect the plant when they reach the stoma. Most varieties of paprika are sensitive to *X. euvesicatoria*.However, less sensitive varieties should be sown and healthy certified seeds are obligatory. However, in field conditions, it regularly appears, especially in areas where paprika is grown in large areas.Copper-based preparations are used to protect peppers, which must be used frequently, and since it acts only in contact, the efficiency is relatively limited (Mojašević et al., 2006). In recent times, Nordox 75 WG based on copper oxide (Cu_2O) has appeared on the market, which acts as a surface bactericide. Unlike other copper-based preparations, the particle size is micronized and is 1.2 μg, with copper hydroxide the particle size is 1.8 a and with copper oxichloride 2.5 μg, which allows it to be better distributed on the sheet, which significantly increases efficiency. Better distribution and contact with bacteria prevents bacteria from swimming and causing infections through the stoma or injuries to the leaf and fruit. Copper is of natural origin, it is also allowed in organic production.However, the frequent application is not desirable because it is a heavy metal and can have a negative effect on earthworms and the environment, which reduces the formation of humus and loses soil quality.In the absence of other effective bactericides, frequent use of copper leads to the formation of resistant strains of *X. euvesicatoria* which has been observed in the USA, Europe and Australia.(Martin et al., 2004). In addition to the use of copper, it is of interest to determine the possible possibility of the action of extracts and enzymes of plant origin such as those isolated from Lactobacillus spp names BioClean and Tidal which are registered as fertilizers and can inhibit effects directly on bacteria or through plants that were the goal of the research.

Material and Methods

The experiment was performed in the experimental field Agricultural Extension Service Sombor PSS Sombor on the variety Bagoly F1 (Hungary), which has elongated fruits, extremely thick flesh with 3-4 chambers. The susceptibility of the cultivar Bagoly to *X.*

euvesicatoria is unknown. Sowing was done on May 23, 2019. The experiment had 4 variants, each variant had 3 repetitions. The experimental unit was a plant and 1 replicate was an area of 11 x 1 m^2. The treatment of paprika was on 26.06, 16.07 and 25.07 2019 and the grade was 5.07., 16.07 and 25.07 2019. Yield measurements were performed during the first and second harvests on August 5 and 31.

The following preparations were used in the experiment
1.Cuproxat SC based on three-base copper sulfate. It belongs to non-systemic fungicides with preventive action (FRAC M1). It is registered for treatments before the conditions for infection are met.
2.Nordox 75 WG based on copper from copper oxochloride. It belongs to the inorganic copper compounds, fungicide with protective action (FRAC M1). It is used for preventive treatments, at the latest until the appearance of the first symptoms.
3.Tidal soluble Concentrate SL, is a formula resulting from a fermentation proces which produces metabolites that are high-performance,biochelants, and the micro-elementsin the formula act as specific coenzymes in the enzymatic processes of the plant.Tidal is specially indicated to avoid or correct symptoms of definciencies of copper, manganese and zink complexed with heptagluconic acid. Registered quantity in vegetables 3l / ha foliar.4. Nutri Bioclean is a solution-SL, a product that strengthens the plant in a way that is not harmful to the environment. It consists of specific enzymes and metabolites of microorganisms. It contains synthetic proteins and organic acids of biological origin. It is obtained by fermentation of the bacterium Lactobacillus, which makes this formulation a biological purifier of plants. Nutri Bioclean creates an unfavorable environment for the development of phytopathogenic fungi and bacteria, and at the same time increases the resistance of plants to their negative effects. Registered quantity in vegetables 2l / ha foliar.
To evaluate the efficacy we used the number of spots per 10 plantsper replicate. The harvest was watered twice on August 5 and August 31, 2019. Author sevaluated the number of healthy, fruits and total yield.
The experiment was set up according to standard OEPP methods.

Results and Discussion

The number of spots of *X. euvesicatoria* in the inspection from July 5 to July 25 at the control increased on the experimental plot. On control, it was increased from 35 to 90 spots. On the BioClean treatment, it was increased from 13 spots to 29, and with Tidal from 18 to 30 spots, which means that the fertilizers failed to prevent infections, but the number of spots was significantly reduced compared to control.
On the Nordox treatment,the number of spots from 17 was reduced to 14 and eventually to 8 spots. Fertilizers Tidal and Beaclean have a supplementary effect on *X. euvesicatoria* as declared by the manufacturer but cannot be used as a bactericide. Copper oxide in the form of Cuproxat had a certain increase in the number of spots in the second grade, but in the third grade, slightly less or 26 was found in relation to the number of spots before treatment.



Photo 2. Healthy paprika plants



Photo 3. Necrotic spots on peppers caused by *X.euvesicatoria* on the lower leaves

Table 1. Intensity and efficacy of the examined plants 05.07.2019.

Variants	Dose l, kg/ha	Treatment time	Examined 30 plants	
			disease intensity %	efficiency preparation %
copper sulfate	3 l /ha	Jun 26	10	26,4
copper oxochloride	2 kg/ha	Jun 26	6,2	54,3
Tidal	2 kg/ha	Jun 26	6,3	53,6
NutriBioclean	2 kg/ha	Jun 26	4,7	65,4
Control	-		13,6	

Table 1 shows the intensity of the disease on 30 plants and the efficiency of the applied preparations, and in the control the intensity of the disease was 13.6%, and in the variants with the applied preparations the intensity of the disease ranged from 4.7 to 10%. The efficiency of the applied preparations in the suppression of bacteriosis ranged from 26.4 to 65.4%. The highest efficiency was achieved in variant number four 65.4% where NutriBioclean fertilizer was applied.

Table 2. Intensity and efficacy of the examined plants 16.07.2019.

Variants	Dose l, kg/ha	Treatment time	Examined 30 plants	
			disease intensity %	efficiency preparation %
copper sulfate	3 l /ha	July 05	9	47,2
copper oxochloride	2 kg/ha	July 05	3,7	78,4
Tidal	2 kg/ha	July 05	5,7	66,6
NutriBioclean	2 kg/ha	July 05	4,3	75
Control	-		17,1	

Table 2 shows the intensity of the disease on 30 plants and the efficiency of the applied preparations, and in the control the intensity of the disease was 17.1%, and in the variants with the applied preparations the intensity of the disease ranged from 3.7 to 9%. The efficiency of the applied preparations in the suppression of bacteriosis ranged from 47.2 to 78.4%. The highest efficiency was achieved in variant number two 78.4% where a fungicide based on the active substance copper oxochloride was applied.

Table 3. Intensity and efficacy of the examined plants 25.07.2019.

Variants	Dose l, kg/ha	Treatment time	Examined 30 plants	
			disease intensity %	efficiency preparation %
copper sulfate	3 l /ha	July 16	3,3	77,8
copper oxochloride	2 kg/ha	July 16	1,02	93
Tidal	2 kg/ha	July 16	3,8	74,3
NutriBioclean	2 kg/ha	July 16	3,9	73,5
Control	-		14,7	

Table 3 shows the intensity of the disease on 30 plants and the efficiency of the applied preparations, and in the control the intensity of the disease was 14.7%, which is significantly higher than the intensity in the variants where it ranged from 1.02 to 3.9%. The efficiency of the applied preparations in the suppression of bacteriosis ranged from 73.5 to 93%. The highest efficiency was achieved in variant number two 93% where a fungicide based on the active substance copper oxochloride was applied.

Table 4.Number of undamaged paprika fruits and yield (kg) after application the products based on copper and NutriLiders fertilizers

Variants	Dose l,kg/ha	Treatment time	Number of undamaged fruits and yield (kg)			
			August 5		August 31	
			Undamaged fruits	Yield (kg)	Undamaged fruits	Yield (kg)
copper sulfate	3 l /ha	Jun 26	688	86	1318	168
	3 l/ha	July 5				
	3 l/ha	July 16				
copper oxochloride	2 kg/ha	Jun 26	767	95	1162	160
	2 kg/ha	July 5				
	2 kg/ha	July 16				
Tidal	2 kg/ha	Jun 26	620	74	1274	159
	2 kg/ha	July 5				
	2 kg/ha	July 16				
NutriBioclean	2 kg/ha	Jun 26	552	81	1222	165
	2 kg/ha	July 5				
	2 kg/ha	July 16				
Control	-		165	19	777	93

Conclusions

Cuproxat SC in the amount of 3 l/ha gave an efficiency of 26 spots and in relation to the Control is 71%. This is expected and the efficiency of copper-based preparations, depending on the size of the inoculum and weather conditions is similar. However, the efficiency of Nordox, based on copper oxide in the new formulation is significantly higher and ranges around 90% or thus a new standard for copper-based preparations is obtained.

Compared to copper-based preparations, *X.euvesicatoria* was similar to Cuproxat and significantly smaller than Control (83). This indicates that Tital and NutriBioClean can be used in alternation with Nordox 75 WG.

In terms of the number of healthy fruits and yields in kg in the first harvest. On August 5, the highest number of fruits and the highest weight was obtained on Nordox 75 WG as expected. In the second harvest, the results show that in relation to copper-based treatments, Tidal and NutriBioclean have an approximate number of fruits and yield differences are obvious in relation to control.Based on this experiment, it can be concluded that Tidal and BioClean act on *X. euvesicatoria* on peppers and can be used in alternation with Nordox 75 XL.

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EFFECTS OF COVERING FIELD AND MEASURES IN CONDITIONS WITH EXTREMELY HIGH TEMPERATURES

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Abstract

Nowadays, the living world is faced with an enormous number of threats and major climate change. This will have significant consequences on the spread of plants, their growth and development. High temperature and stress in plants significantly alter the life cycles of plants. In order to reduce the negative impact of high temperatures, authors set up experiment with peppers and tomatoes at the Agriculture advisory service "Sombor" field in West Bačka District of the Autonomous Province of Vojvodina (Serbia) during two years 2017 and 2018. We set up 7 variants (control 1, control 2, black foil with plants, black foil with hay, black foil with straw, soil without foil with hay, soil without foil with straw). During the test we measured the temperature every day from 10 July to July 31 between 08:00 and 16:00 (08:00,10:00,12:00,14:00,16:00). We measured the temperature of the soil with a digital thermometer, the temperature above the surface of the soil and measurement using an automatic weather station. The results in 2017 and 2018 showed significant reductions in soil temperature in straw and hay variants relative to controls. In addition to measuring temperature, we also measured the results of tomato and pepper yields, where variants with straw and hay significantly increased the yield. This way of soil formation is one of the measures to overcome the problems that arise in extremely high temperatures.

Keywords: *high, temperatures, measuring, foil, straw, hay.*

Introduction

The last decades of the 20th century were marked by the growing interest of the scientific public in climate change and the consequences that this phenomenon can cause in the whole society (IPCC, 2001). Global warming has today become one of the major problems for scientists working on climate change. With the development of industry in 1750, a larger emission of various gases into the atmosphere began. The main gas responsible for the greenhouse effect is carbon dioxide. Today, the percentage of this gas in the atmosphere is much higher than in the last 30 years, and as a result, the temperature has increased. The twenty warmest years recorded in history have occurred in the last 22 years (IPCC, 2018). Global warming, acid rain, ozone depletion and rising sea levels are direct recent air pollution. Many living beings suffer the direct consequences of increasing the concentration of harmful materials in the air. High temperature strongly affects the intensity of transpiration. This has a direct impact on water diffusion and on increasing the degree of water pressure (Ilic, 2007). Plants lose chlorophyll and change color, their tissues and organization are gradually invented, the processes of photosynthesis and growth are stopped, and eventually drying and death occur (Lilić, 2011). The most well-known damage caused by exposing plants to too high temperatures is heat stroke. It occurs when high temperatures occur simultaneously with dry air and warm and dry wind. Hot days are usually those with a maximum temperature of 30 ° C or higher, and days with warm nights are those with a minimum temperature of 20 ° C (Ilin, 2017). In contrast to summer, changes in maximum air temperature are much less pronounced during winter (Lalić, 2017).

Vegetable plants, which are grown in dry farming, lower the temperature of the leaves exclusively through transpiration. In such plants, the leaf temperature in the middle of the day, when the plants are sunny, easily rises 4-50 ºC above the air temperature, when the water deficit in the soil causes partial closure of the stoma or when high relative humidity reduces the possibility of cooling by transpiration (Ilin,2011). Most tissues of higher plants cannot survive long-term exposure to temperatures above 45 ° C, especially when they are in a phase of accelerated growth (Falik, 2009). In order to avoid and reduce the consequences of high temperatures on plants, authors set up an experiment with different variants of coveringon the experimental field "PSS Sombor".

Material and Methods

On the experimental field "PSS Sombor" in Serbia, authors set up a test of covering with pepper and tomatoes for two years (2017 and 2018) in 7 variants: 1 - Control (bare soil without plants), 2 (control, bare soil without black foil with plants), 3 (black foil with plants), 4 (black foil covered with hay), 5 (black foil covered with straw), 6 (land without black foil covered with hay), 7 (land without black foil covered with straw).



Figure 1. Variant 1 (control, bare soil without plants)



Figure 2. Variant 2 (control, bare soil without black foil with plants)



Figure 3. Variant 3 (black foil with plants)



Figure 4. Variant 4(black foil covered with hay)



Figure 5. Variant 5(black foil covered with straw)



Figure 6. Variant 6 (land without black foil covered with hay)



Figure 7. Variant 7 (land without black foil covered with straw)

To measure the soil temperature, authors used a digital TFA thermometer in the temperature measurement range from -40 ºC to +200 ºC, with a probe length of 125 mm, waterproof. To measure the air temperature at a plant height of 20 cm from the ground, we used a digital TFA thermometer in the measurement range from -20 ºC to +70 ºC, waterproof (Fig. 8). To measure the air temperature, authors used an automatic meteorological station (Fig 9) located on the experimental field PSS "Sombor". Before planting the plants, we stretched a thin, elastic black mulch foil.



Figure 8. TFA digital thermometers



Figure 9. Automatic meteorological station

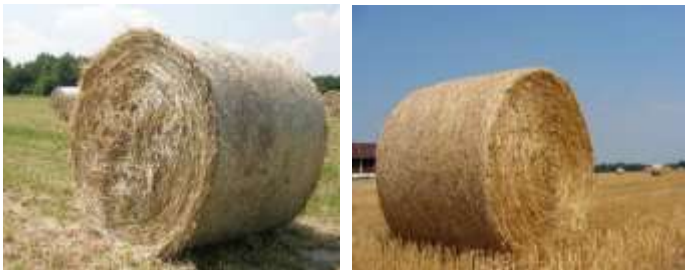


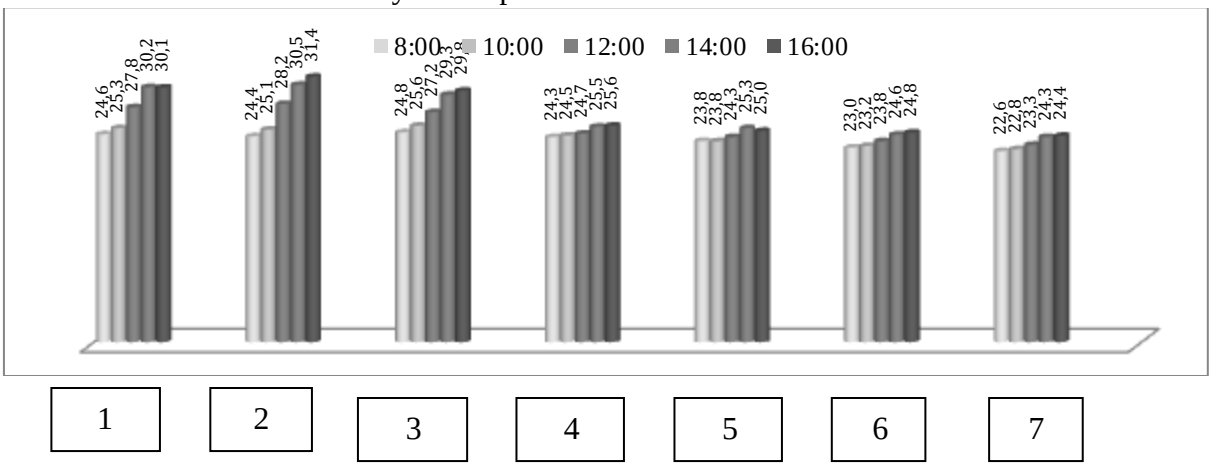
Figure 10 and 11. Bales of hay and straw

Authors set up a demonstration plot with pepper of 1.25 m^2 and with tomatoes of 2.0 m^2. We measured the temperature from July 10 to July 31 in the time regime from 8:00 to 16:00. We used a roll bale of hay and straw bought from local producers in the amount of 5 kilograms per square meter. The temperature was measured five times during the day. For each variant, the soil and air temperature were measured and the obvious data on the air temperature at the meteorological stations. After measuring a certain amount of straw and hay, we put it on experiments with tomatoes and peppers. What is important is that the plants are well "tucked" under straw and hay without empty space.

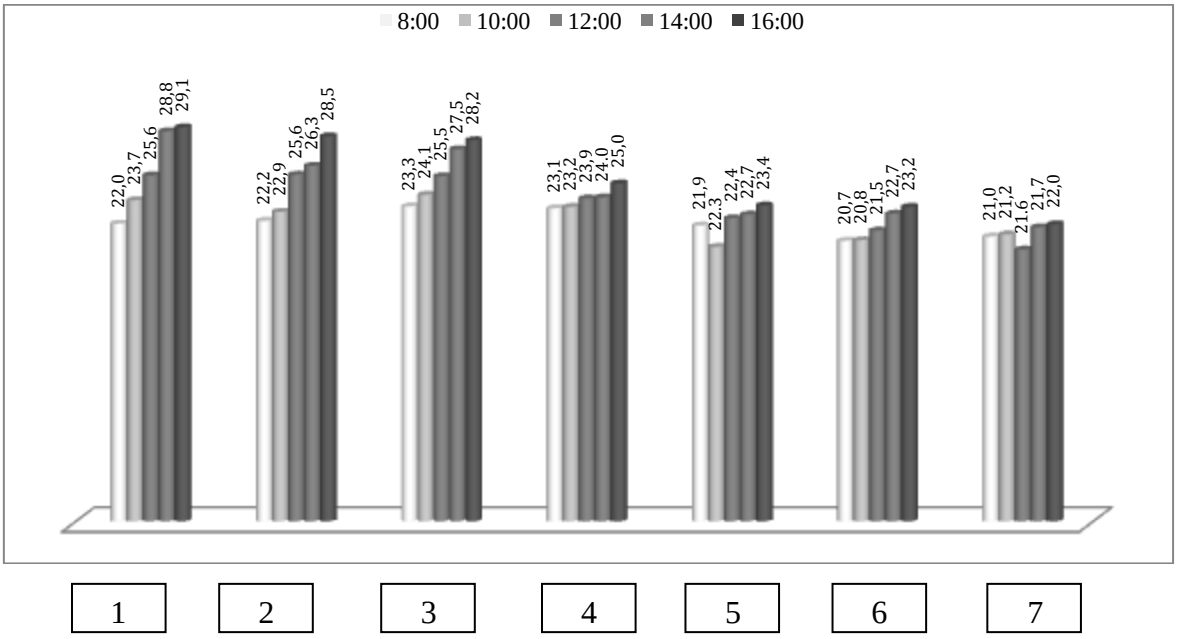
Results and Discussion

The average soil temperatures in the 2017 experiment with pepper showed that the treatments with hay and straw (with and without black mulch foil) have a positive effect on reducing the soil temperature from 3 to 5 ºC, depending on the variant compared to the control (Graph 1, 2). The results of spreading with straw and hay gave similarly good results in the experiment with tomato, where authors obtained a decrease in soil temperature also from 3 to 5 ºC.

Graph 1. Average soil temperatures in the experiment of pepper with spreading from the 10th to 31st July in the period from 08:00 to 16:00 in 2017

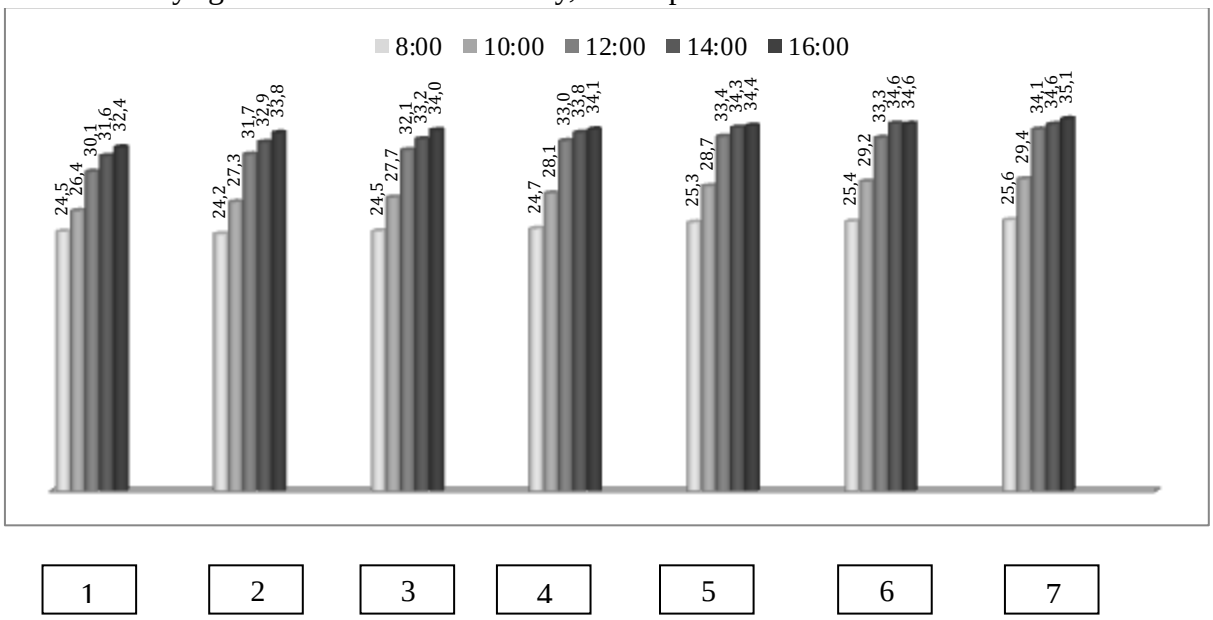


Graph 2: Average soil temperatures in the experiment of tomato with spreading from 10th to 31st July in the period from 08:00 to 16:00 in 2017.



The average air temperatures in the plant zone (at a height of 20 cm) showed that the spread variants have no effect on reducing the air temperature in the plant zone (Graph 3).

Graph 3: Average air temperatures in the plant zone (at a height of 20 cm) in the experiment with laying from the 10th to 31st July, in the period from 08:00 to 16:00 in 2017



In addition to air and soil temperature, we also measured the yield of pepper, and we obtained a variant of black foil with hay (42.7 t/ha), an increase in yield of 17.1 t/ha compared to the control (25.6 t/ha) (Table 1). Also, all other variants of black foil with straw (32.8 t/ha), land without foil with hay (35.5 t/ha) and land without foil with straw (29.8 t/ ha) had a statistically significant increase in yield compared to control (25.6 t/ha) (Table 1).

Table 1. Results of micro-experiments of table pepper and the percentage of yield damage with different variants of spreading in 2017

Repetition	Plant control		Black foil with plants		Black foil with hay		Black foil with straw		Land without foil with hay		Land without foil with straw	
	Yield t/ha	% damage	Yield t/ha	% damage	Yield t/ha	% damage	Yield t/ha	% damage	Yield t/ha	% damage	Yield t/ha	% damage
I	31.2	8.8	23.8	20.2	25.0	23.9	24.8	13.6	23.1	9.5	13.6	22.2
II	22.3	0.0	26.7	6.4	45.4	9.2	38.1	18.4	50.3	9.3	36.6	9.0
III	23.3	7.5	35.2	10.8	57.8	11.5	35.6	21.7	33.3	9.3	27.3	16.1
Average	25.6	8.7	28.5	12.4	42.7	14.8	32.8	17.9	35.5	9.3	29.8	15.7

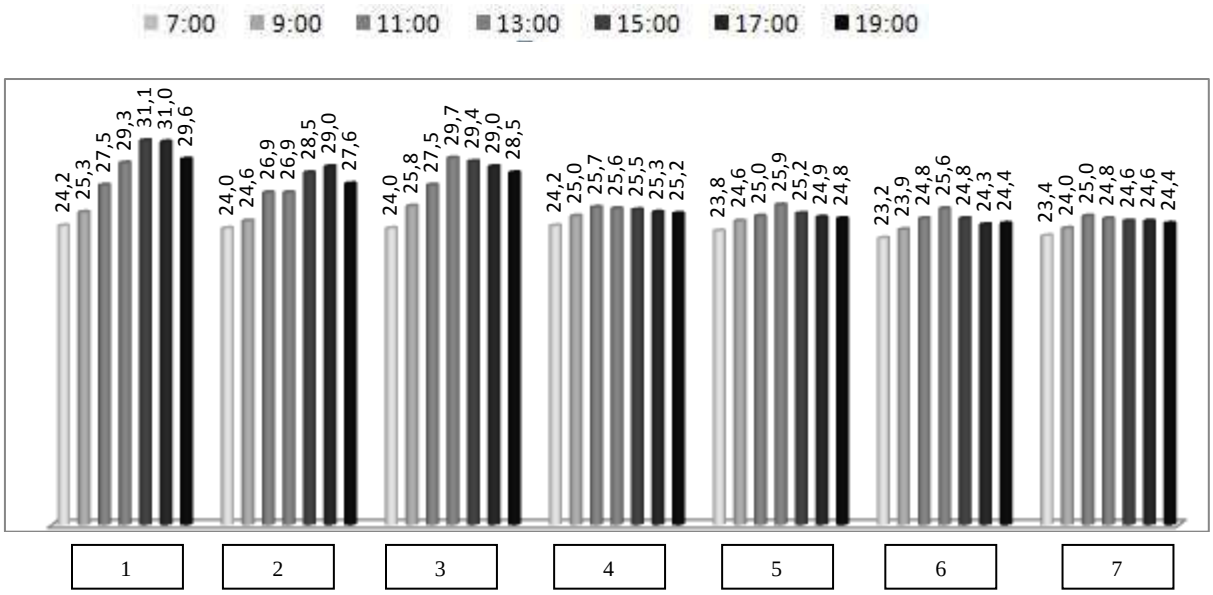
Also, in the experiment with tomato, the best variant was land without foil with hay 136.0 t/ha in relation to the control 82.7 t/ha (Table 2). Other variants with spreading showed a statistically significant increase in yield of black foil with hay (105.7 t/ha), black foil with straw (131.3 t/ha) and land without foil with straw (124.8 t/ha) compared to the control (82.7 t/ha).

Table 2. Results of micro-experiments of tomatoes and the percentage of yield damage with different variants of spreading in 2017

Repetition	Plant control		Black foil with plants		Black foil with hay		Black foil with straw		Land without foil with hay		Land without foil with straw	
	Yield t/ha	% damage	Yield t/ha	% damage	Yield t/ha	% damage	Yield t/ha	% damage	Yield t/ha	% damage	Yield t/ha	% damage
I	64.5	29.5	89.1	22.8	98.0	29.8	89.5	27.6	96.0	22.8	76.0	22.0
II	63.5	22.3	80.0	21.8	100.5	24.6	149.0	34.3	149.0	19.5	146.5	27.8
III	120.0	60.1	108.0	27.8	118.5	22.0	155.5	24.3	163.0	19.6	152.0	25.6
Average	82.7	37.3	92.4	24.1	105.7	25.5	131.3	28.7	136.0	20.6	124.8	25.1

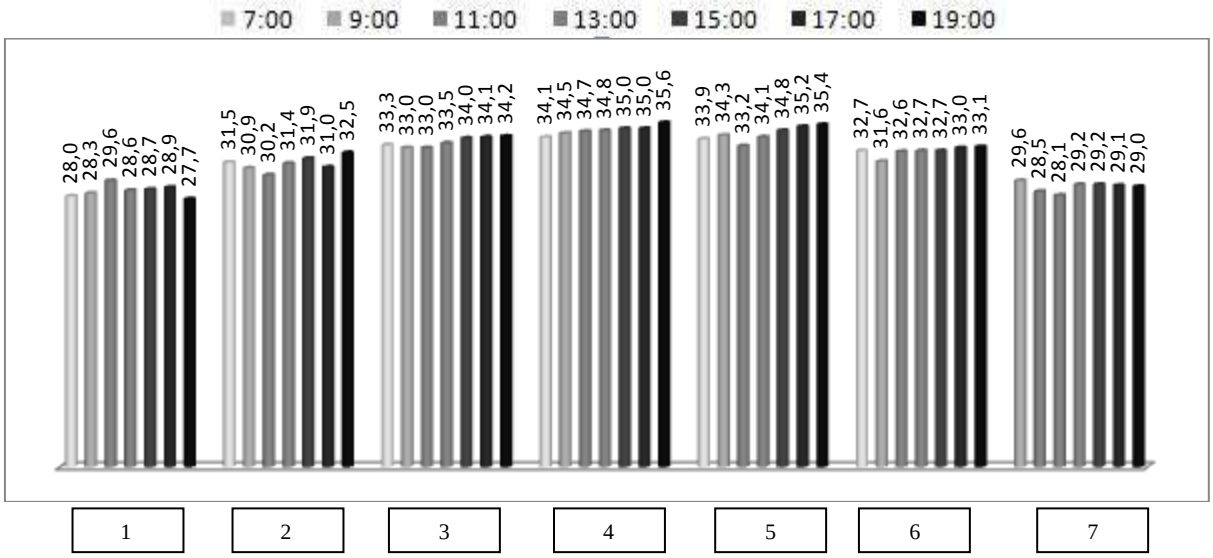
During the second year in 2018, from July 13 to August 30, in the period from 07:00 to 19:00 we measured the soil temperature and air temperature. In the experiment with pepper on all variants with spreading, we obtained a significant reduction of soil temperature from 5 to 7 ºC in relation to the control (Graph 4). We had also positive results on the variant of soil without foil with straw, we obtained a reduction of soil temperature from 2 to 4 ºC in relation to the control.

Graph 4: Average soil temperatures in variants with spraying in the pepper experiment in the period from July 13 to August 30 in 2018 year



Compared to 2017, where we did not have a significant increase in air temperature in the plant zone in 2018, except for the variant of soil without foil with straw, we had an increase in temperature of 2 to 4 ºC in the variant with spreading compared to the control.

Graph 5: Average air temperatures in variants with spraying in the pepper experiment in the period from July 13 to August 30 in 2018



In the variant black foil with hay on pepper we had an increase in yield of 2.5 t/ha compared to the control (31.2 t/ha), while in the experiment with tomatoes on the variant black foil with straw (50.0 t/ha) we had a statistically significant increase in yield compared to control (36.4 t/ha). We had a statistically significant increase in yield on the variants of land without foil with hay (42.8 t/ha) and land without foil with straw (39.8 t/ha).

Table 3: Results of micro-examination of table pepper and percentage of yield damage with different variants of laying in 2018.

repetition	plant control		black foil with plants		black foil with hay		black foil with straw		land without foil with hay		land without foil with straw	
	yield t/ha	% damage	yield t/ha	% damage	yield t/ha	% damage	yield t/ha	% damage	yield t/ha	% damage	yield t/ha	% damage
I	26.1	25.4	30.3	29.7	29.7	24.5	39.7	25.8	30.9	20.2	33.3	21.0
II	31.1	17.2	26.2	36.5	23.1	27.3	25.6	27.2	37.3	26.2	28.5	30.0
III	36.5	25.4	37.3	25.4	48.3	29.1	16.5	25.4	24.5	32.9	31.8	19.1
average	31.2	22.7	31.3	30.5	33.7	27.0	27.3	26.1	30.9	26.4	31.2	23.4

Table 4: Results of tomato microexamination and percentage of fruit damage with different variants of laying in 2018.

repetition	plant control		black foil with plants		black foil with hay		black foil with straw		land without foil with hay		land without foil with straw	
	yield t/ha	% damage	yield t/ha	% damage	yield t/ha	% damage	yield t/ha	% damage	yield t/ha	% damage	yield t/ha	% damage
I	18.2	37.0	38.0	32.7	36.2	34.3	63.5	29.2	43.8	34.4	48.1	37.3
II	46.9	25.8	39.5	23.1	32.8	36.6	40.6	19.2	56.0	28.0	37.3	24.8
III	44.2	38,4	31.7	20.6	30.8	26.3	45.8	23.2	28.6	29.2	33.9	25.7
average	36.4	33.8	36.5	38.3	25.5	32.4	50.0	23.8	42.8	30.6	39.8	29.3

Compared to 2017, in the experiment with tomatoes in 2018, we recorded an increase in temperature in the plant zone in all variants except for the variant of soil without foil with straw in relation to the control.

Graph 6. Average air temperatures in the plant zone in variants with spreading in the tomato experiment in the period from July 13 to August 30 in 2018 years

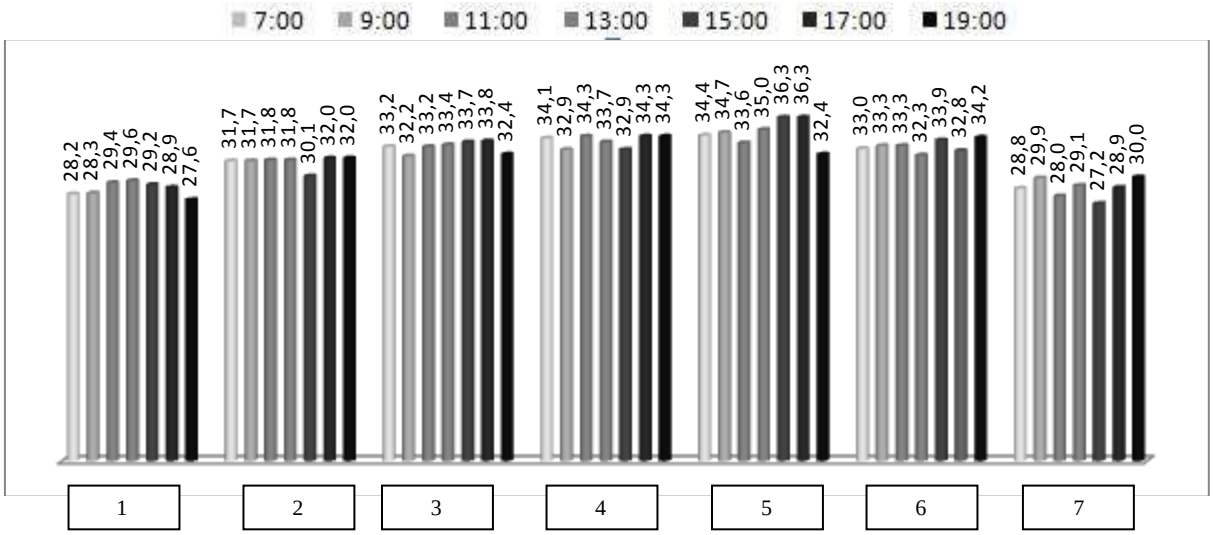
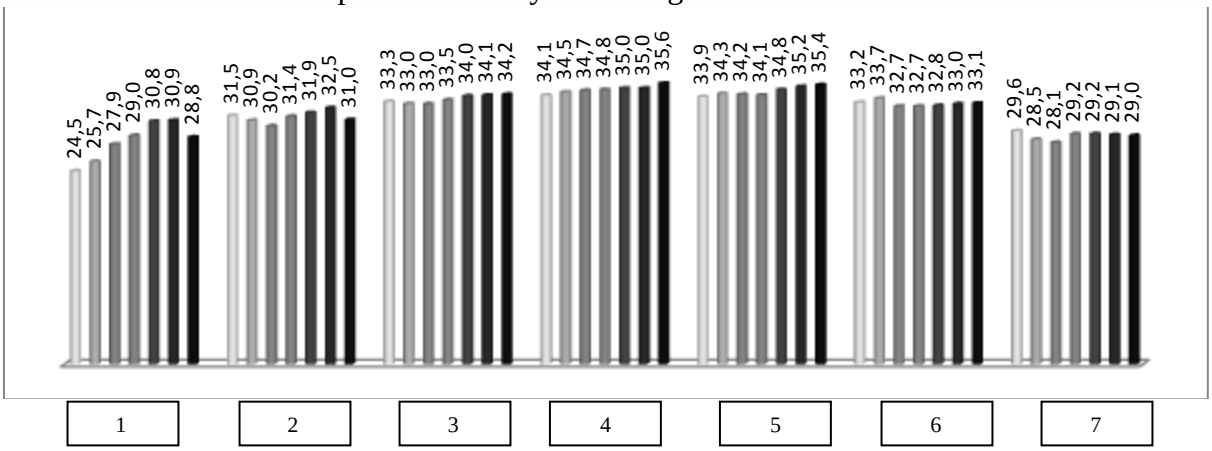


Figure 7: Average soil temperatures in variants with spreading in the tomato experiment in the period from July 13 to August 30 in 2018



Conclusions

The influence of temperature on the plant species depends on the characteristics and requirements of the variety. Stress caused by high temperatures in the open field can be significantly reduced by applying agronomic approaches such as deep tillage, quality pre-sowing preparation, cultivation during vegetation, irrigation and a good selection of genotypes tolerant of high temperatures and drought. Our primary goal was to try to lower the temperature of the soil. If we compare the results of the first years and secondly years of research, we can clearly see that the variants of covering with straw and hay had excellent results in lowering the soil temperature. Also, in addition to lowering the soil temperature, we obtained and increased the yield on some variants of 50 t/ha. Therefore, the data can conclude that spraying has multiple uses in protecting plants from the effects of extreme temperatures.

The advantages of spreading the soil in the production of vegetable crops, increased yields and quality yields, a positive impact on the physical and biological properties of the soil as well as preventing the growth of weeds. In addition to the advantages, we also have the disadvantages of spreading the soil, such as additional costs, unpleasant odors as a result of the work of anaerobic bacteria, excessive water retention in the root area. Based on long-term results, we can conclude that the measures taken to prevent plant deaths caused by high temperatures have had a positive impact on reducing air temperature and increasing yields from year to year.

Since the climate is changing significantly and we have fewer rainy days and more warm periods, we will continue with research to find more agro-technical solutions to reduce the impact of high temperatures and reduce the negative impact of high temperatures on plants.

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APPLICATION OF UNMANNED AERIAL VEHICLE TECHNOLOGY IN PLANT DISEASES DETECTION

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Abstract

The application of unmanned aerial vehicles (UAV) in corporation with remote sensing technology is a very promising branch of technology nowadays, and it offers the potential to collect detailed spatial information in real time at relatively low cost. UAV with remote sensing (RS) data have been frequently cited as a rapid, safe, non-destructive, and cost-effective tool for plant disease detection in different contexts and in different types of plant species.UAV technology has been used in agriculture with image processing technologies for variable purposes including; detection, monitoring, and identification of weeds and plant diseases, estimating the expected yield of agricultural products in their various stages of development. Accurate estimates of disease symptoms, the level of disease severity, and the negative effects of diseases on the quality and quantity of agricultural products are important for field crops, horticulture, plant breeding, and for improving the efficiency of fungicide, herbicide, and insecticide, as well as for basic and applied plant research. Since pest control usually consists of taking decisions based on; the level of infestation, the development stage of the plant, timely assessments of plant disease occurrence and disease spread, In this study; studies in crop disease detection with unmanned aerial vehicles technology and remote sensing as a challenging area that can have significant economic and environmental impact on crop disease management were provided, traditional disease detection methods were discussed, comparison between the traditional methods that used for crop disease detection and unmanned aerial vehicles remote sensing based methods, limitations faced by unmanned aerial vehicles technology, overview of current sensor technologies used for the automated detection and identification of plant diseases, and the benefits obtained as a result of using these new technologies were discussed.

Keywords: *Remote sensing; UAV; Disease detection; sensor.*

Introduction

The applications of unmanned aerial vehicles (UAVs), or drones, have generated a large interest across many fields. UAVs offer the potential to collect information in real time at relatively low cost and are being used intensively in conservation and ecological research (Fornace et al. 2014).Remote sensing (RS) data has been frequently cited as a rapid, non-destructive, and cost-effective tool for plant disease detection in different contexts and with different plant species (Kazmi and Usery 2001). Indeed, many diseases can affect plant physiology, leading to modifications on biophysical and biochemical characteristics of tissues and differences in leaf optical properties of plant leafs (Carter and Knapp 2001).
Accurate estimates of disease incidence, level of disease severity, and the negative effects of diseases on the quality and quantity of agricultural products are important for all field crops, horticulture, and for improving the effect of fungicide as well as for basic and applied plant

research. Timely assessments of plant disease occurrence and disease spread are, in particular, the basis for planning targeted plant protection activities in the field and greenhouse production. Common methods for the diagnosis and detection of plant diseases include visual plant disease estimation by human raters, microscopic evaluation of morphology features to identify pathogens, molecular, serological, and microbiological diagnostic techniques (Bock et al. 2010). Ward et al. (2004), stated that microbiological methods exist for pathogen isolation on selective, artificial media and molecular and serological methods can be readily applied for the diagnosis and detection of a pathogen. These methods are used by plant protection services and in both research and industrial development. Recently; sensing techniques take their place in automatic detection and identification of plant reactions.The main aim of this review is to provide; studies in crop disease detection with unmanned aerial vehicle technology as a challenging area that can have significant economic and environmental effect on crop disease management in different stage of development.

Recent researches in plant disease detection using sensor technology

A large number of more recent works in different patho-systems using different types of highly sensitive sensors (Fig. 1) and multiple data analysis programs have been published and a few examples are provided in this part as well as summarized in Table 1.

Albetis et al. (2017) in their study; evaluate the feasibility of discriminate the Flavescencedorée symptoms in red and white cultivars from healthy vine vegetation using UAV multispectral imagery. Spectral signatures of healthy and symptomatic plants were studied using a set of 20 variables computed from the UAV multispectral images (spectral bands, vegetation indices and biophysical parameters) using univariate and multivariate classification approaches. The most significant results were achieved with red cultivars.

Dash et al. (2017) simulated a disease outbreak in mature Pinusradiata D. Don trees using targeted application of herbicide. The objective of their study was to acquire a time-series simulated disease expression dataset to develop methods for monitoring physiological stress from a UAV platform. Time-series multi-spectral imagery was acquired using a UAV flown over a trial at regular intervals. The results showed that multi-spectral imagery collected from a UAV is useful for identifying physiological stress in mature plantation trees even during the early stages of tree stress. They found that physiological stress could be detected earliest using data from the red edge and near-infrared bands. But when compared with the previous findings, red edge data did not offer earlier detection of physiological stress than the near infra-red data. Vega et al. (2015) determine the capability of an UAV system with a multispectral sensor to acquire multi-temporal images during the growing season of a sunflower crop. Measurements were made at different times of the day and with different resolutions to estimate the normalized difference vegetation index (NDVI) and evaluate its relationship with several indices related to crop status with the aim ofgenerating useful information for application to precision agriculture techniques. They found that the linear regressions between NDVI and grain yield, aerial biomass and nitrogen content in the biomass were significant at the 99% confidence level, except during very early growth stages, they found also that the image acquisition time, classification process, and image resolution had no any effect on the results. Di Gennaro et al. (2016) suggest a methodology to investigate the relationships between high-resolution multispectral images (0.05 m/pixel) acquired using an UAV, and GLSD foliar symptoms monitored by ground surveys. They stated that themethod showed high correlation between Normalized Differential Vegetation Index (NDVI) acquired by the UAV and GLSD symptoms, and discrimination between symptomatic from asymptomatic plants.

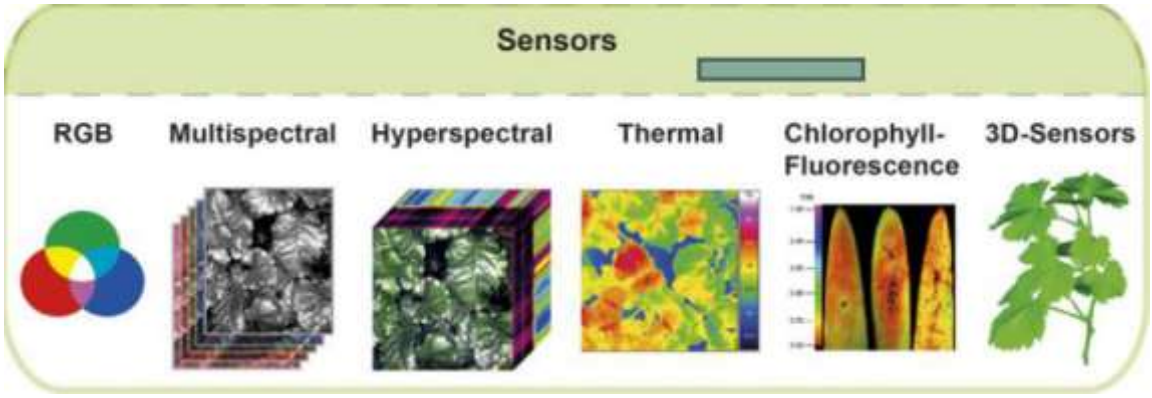


Fig1. Overview of current sensor technologies used for the automated detection and identification of host-plant interactions (from single cells to entire ecosystems) (Mahlein 2016).

Garcia-Ruiz et al. (2013) present a new approach of high-resolution aerial imaging for HLB (or citrus greening disease) detection using a low-cost, low-altitude remote sensing multi-rotor unmanned aerial vehicle. A multi-band imaging sensor was attached to a UAV that is capable of acquiring aerialimages at desired resolution by adjusting the flying altitude. Moreover, the results achieved using UAV-based sensors were compared with a similar imaging system (aircraft-based sensors) with lower spatial resolution. Six spectral bands were used in the study (from 530 to 900 nm) with seven vegetation indicesderived from the selected bands. For analysis stepwise regression was used to extract the featuresfrom UAV-based and aircraft-based spectral images. As a result, 710 nm reflectanceand NIR-R index values were found to be significantly different between healthy and HLB-infected trees.

Table1. Examples of plant diseases assessed by optical sensor technology

Sensor	Crop	Scale	Disease	Reference
Multispectral imagery(visiblea nd near-infrared domain)	grapevines(*Vitisvini feraL.,Vitaceae*)	Field	Flavescencedorée	(Albetis et al. 2017)
RGB	Cotton	Leaf	Bacterialangular (Xanthomonascampe stris)Ascochytablight (Ascochytagossypii)	(Roscher et al. 2016)
Multi-spectral imagery	Pinusradiata D. Don trees	Ecosystem	Physiological stress	(Dash et al. 2017)
Multispectral imagery	Potato	Field	Hail damage	(Zhou et al. 2016)
Spectroscopy-the visible and near-infrared (NIR)	Soybean	Leaf	Soybean cyst nematode	(Bajwa, Rupe, and Mason 2017)

Sony EXMOR sensor	Soybean	Field	Soybean foliar diseases	(Tetila et al. 2017)
Thermal imagery	Opium poppy	Single crop	Downy mildew *Peronospora arborescens* (Berk.)	(Calderón Madrid et al. 2014)

Comparison between traditional agricultural management and optical sensing techniques

Traditional agricultural management practices assume that the parameters in crop fields are completely homogeneous, so pesticides are always applied at equal quantities, regardless of the presence or absence of an actual infection. In general, the occurrence of plant diseases depends largely on specific environmental factors that differ from one pathogen to another. Diseases often show a heterogeneous distribution in the fields which makes conventional detection methods (based on characteristic plant symptoms; e.g., lesions, blight, galls, tumors, cankers, wilts, rots, or damping-off) economically ineffective. Optical sensing techniques are useful in identifying the primary disease foci and areas that differ in disease severity in fields as illustrated in the literature (Vega et al. 2015; Behmann et al. 2015).

In combination with data analysis methods, these techniques can be used for targeted pest management programs in sustainable crop production. targeted applications of pesticides according to precision crop protection strategies results in, potential reduction in pesticide use as well as reduce the economic expense and ecological impact in agricultural crop production systems (Gebbers and Adamchuk 2010). Traditional detection methods are time consuming methods and need experienced, well-developed skills in diagnosis and disease detection, in addition to presence of a high rate of errors during diagnosis. In 1936, Riker and Riker emphasized the difficulties in diagnosing and detecting plant diseases. They gave an overview of the strengths and limitations of existing methods and concluded that: "We need better methods for diagnosis; none of the methods given are to be considered as 'standardized'. To think of them in such a way would put an end to efforts of improvement. They are useful only until better procedures can be developed." This confirms the necessity of applying new technologies in plant disease detection.

Recently intensive research has identified new sensor-based methods for detection, identification, and quantification of plant diseases (Mahlein et al. 2012; Sankaran et al. 2010). These sensors assess the optical properties of plants within different regions of the electromagnetic spectrum and beyond the visible range like NIR reflectance (West et al. 2003).

Limitations

The use of remote sensing technology for crop disease detection requires high spatial and spectral resolution. Thomas et al. (2017) in their study stated that; the current satellite-based imagery has limited application in crop management due to the low spatial and spectral resolutions provided and the long revisit periods.High spatial resolution imagery acquired in the visible and near-infrared regions is relatively feasible with current airborne and satellite sensors. By contrast, thermal imaging is still limited to medium-resolution sensors due to the technical limitations of micro-bolometer technology.

The common limitation of optical sensors is the complexity of the collected data. In order to be able to efficiently use optical sensor data to detect diseases, advanced data analysis and statistical methods are essential. Data collected by optical sensors has to include important parameters(i) the detection of a disease at early points in time, (ii) the differentiation among different diseases, (iii) the separation of diseases caused by a biotic stresses, and (iv) the

quantification of disease severity (Thomas et al. 2017). The data has to include several important factors: (i) the detection of a disease at early points in time, (ii) the differentiation among different diseases, (iii) the separation of diseases caused by a biotic stress, and (iv) the quantification of disease severity. These parameters need to be evaluated with a level higher or equivalent to the accuracy attained with standard assessment method.

Conclusion

The worldwide production of agricultural products is less than the actual demand; hence there is a need to manage the agricultural production efficiently. The application of UAV-based remote sensing has increased the opportunities in agricultural production management and precision crop protection, including the detection, monitoring, and identification of weeds and plant diseases. Unmanned aerial vehicles in combination with computer vision and machine learning methods have played a major role for automatic measurement and classification of the remote images. Here, studies in crop disease detection with unmanned aerial vehicle technology as a challenging area that can have significant economic and environmental effect on crop disease management in different stage of development were provided. Accurate results had been obtained in previous studies, but new and automated methods with high sensitivity, specificity, and reliability are still needed to improve disease detection.

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PRESENCE OF AFLATOXIN M1 IN MILK IN SERBIA IN 2017

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Abstract

Aflatoxins are the most widespread mycotoxins primarily produced by fungi of the genus *Aspergillus* such as *A. flavus* and *A. parasiticus*, infecting grains during storage, spaces, nuts, milk and animal feed. Even in low concentrations, they are highly toxic. Major forms of aflatoxins include B1, B2, G1 and G2. Mammals that ingest AFB1 contaminated food eliminate amounts of the main hepatic metabolite known as „milk toxin" or aflatoxin M1 (AFM1) via milk. In this study, a total of 368 samples of raw, pasteurised and UHT milk was examined in 2017. All samples were analyzed for AFM1 by competitive enzyme-linked immunoassay, RIDASCREEN® Aflatoxin M1. AFM1 was detected in 50 out of 56 samples of raw milk (89.28%), at levels ranging from 0.04 to 2.10µg/l. Of 102 samples of pasteurized milk, 69 were contaminated by AFM1 (67.65%), at levels ranging from 0.03 to 0.199µg/l. Finally, of 210 UHT milk samples AFM1 was detected in 107 (50.95%), at levels ranging from 0.025 to 0.126µg/l. In Republic of Serbia, maximum residue limit of AFM1 is 0.25µg/kg. These results suggest that number of milk samples contaminated with AFM1 is not negligible. In addition, the level of contamination was the highest in raw milk. The occurrence of aflatoxins in raw milk and commercially available milk is one of the most serious problems, as milk is a key source of nutrients for human. Therefore, continuous monitoring over milk is necessary.

Key words*: Aflatoxin M1, milk, ELISA.*

Introduction

Aflatoxins (AFs) are one of the most investigated group of mycotoxins, which can be found as contaminants in different types of food and feed (Kos *et al.*, 2015). They are considering to be the most toxic and carcinogenic of the naturally occurring mycotoxins (Whitaker, 2006).
AFs are secondary metabolites mainly produced by fungi of genus *Aspergillus* (*A.flavus*, *A.nomius*, *A.parasiticus*) in agricultural products from tropical and subtropical climatic conditions like those existing in Latin American, Asian and African countries, southern regions of US and certain parts of Australia (Devegowda *et al.*, 2009).
Although, risk of aflatoxin contamination is generally higher in warm and humid climatic conditions, as a result of the very hot and dry seasons during the last 15 years, 'higher-temperature mycotoxins' became a significant problem in countries where this had previously been uncommon, such as most of the European countries, including Serbia (Fakhri *et al.*, 2019; Milićević *et al.*, 2019, Serraino *et al.* 2019).
Among approximately 18 identified AFs, aflatoxin B1 (AFB1) is the most common as well as highly toxic, mutagenic, teratogenic and carcinogenic compound (Kos *et al.*, 2014).
The hydroxylated metabolite of aflatoxin B1 (AFB1) that can be found in milk and in other dairy products in consequence of their presence in cattle feed is aflatoxin M1 (AFM1) (Prandini *et al.*, 2007; Trevissani *et al.*, 2014).
If ruminants are fed with contaminated feed, the AFB1 consumed by the animals is partly degraded by the forestomach before reaching the circulatory system. The remaining part is

transformed by the liver into monohydroxy derivative forms such as AFM1 (mainly), and other hepatic metabolites such as AFM2, AFM4, aflatoxicol and others (in smaller quantities). Afterward, it is being secreted into the milk through the mammary glands (Frazzoli *et al.*, 2017). AFM1 residues in milk are variable percentage (0.3-6.2%) of AFB1 ingested (Galvano *et al.*, 2009).

Depending on the level of contaminated feedstuff are used, AFM1 can be detected in milk 6-24 h after first intake of AFB1, while its concentration decreases to an undetectable level 72 h after the initial intake is stopped (Kos, 2015).

Until 2002, AFM1 was classified in 2B group as a possible carcinogen for human. However, based on numerous studies regarding carcinogenic, teratogenic, genotoxic and immunosuppressive effect, AFM1 was reclassified into first group (Kos *et al.*, 2014).

Considering that milk is a highly nutritious food containing many macro- and micronutrients that are essential for the growth and maintenance of human health, the occurrence of AFM1 in milk is one of the most serious problems (Iqbal *et al.*, 2015, Kerekes *et al.*, 2016).

Therefore, the objective of this study was examination and determination of the presence of AFM1 in raw milk, pasteurized milk and ultra high temperature processed milk (UHT milk) in order to determine the levels of contamination.

Materials and Methods

Collection of samples

Present study examined 368 samples of different types of milk. All samples were collected during 2017, as a part of the food safety control. First group od milk samples included 56 samples of raw milk. Second group included 102 samples of pasteurized milk and finally, third group were 210 samples of UHT milk. Before analysis, the samples were stored at regrigerator (4-6ºC), protected from light.

Sample preparation

All collected samples were prepared and analyzed in accredited laboratory for testing food and feed safety „Jugoinspekt Beograd". Milk samples were prepared according manufacturer's instructions. Prior to analysis, 20 mL of each milk sample was centrifuged for 10 min at 3000 × g.

The upper creamy layer was removed by aspirating through a Pasteur pipette. Skimmed milk (defatted supernatant) was used in the test (100µl per well) plate.

Aflatoxin analysis and instrumental conditions

Determinaton and quantitive analysis of aflatoxin M1 was done by Enzyme Linked ImmunoSorbent Assay (ELISA) method, using RIDASCREEN Aflatoxin M1 (R-Biopharm) test kit. Analysis were performed according to manufacturer's instructions (RIDASCREEN Aflatoxin M1 Art.No.:1121). The measurement is made photometrically at 450 nm. The absorbance is inversely proportional to the aflatoxin M1 concentration in the sample. Multiskan FC microplate reader with absorbance range 0 - 6.000 A was used. Normal reading mode was used with reading speed t = 13 s. The absorbance is inversely proporcional to the aflatoxin M1 in the sample . Special software Rida® Soft Win (Art. No. Z9999, R-Biopharm, Germany) was used for the evaluation of enzyme immunoassays. The analytical quality of the ELISA method was assured by determination of spiked samples as well as by participation in proficiency testing scheme (RVEP 170034 milk residues). Recovery for AFM1 was 105%. Using method was validated (LOD=0.01µg/l, Recovery=93%).

Results and Discussion

The results on occurrence of aflatoxin M1 in raw milk, pasteurized milk and UHT milk are represented in Table 1 and Chart 1.

Aflatoxin M1 was detected in 50 out of 56 raw milk samples (89.28%), at levels ranging from 0.04 to 2.10 µg/l. Of 102 samples of pasteurized milk, 69 were contaminated by aflatoxin M1 (67.65%), at levels ranging from 0.03 to 0.199µg/l. Finally, of 210 UHT milk samples AFM1 was detected in 107 (50.95%), at levels ranging from 0.025 to 0.126µg/l. In 14.67% of samples, maximum EU level for AFM1 in milk (0.05 µg/kg) was exceeded which is significantly lower frequency of AFM1 contamination than reported by Jakšić *et al.* (2017) for the period 2013-2015, where 73.3% of the samples exceeded concentration of AFM1 of 0.05 µg/kg in 2013, 39.4 % in 2015 and 28.6% in 2015. However, this result is in accordance with published results reported by the same authors for the 2016, where 13.9% of the samples exceeded concentration of AFM1 of 0.05 µg/kg. This graduate decline could be explained by the fact that since 2014 there was no significant contamination of corn with aflatoxins (Nešić *et al.*, 2015).

Table 1. Occurrence of aflatoxin M1 (AFM1) in raw milk, pasteurized milk and UHT milk

Commodity	No. of positives/total	Average value[1]	Max value	Interval of concentration (contaminated samples)
Raw milk	50/56	0.167	2.1	0.04-2.10
Pasteurized milk	69/102	0.047	0.199	0.03-0.199
UHT milk	107/210	0.024	0.126	0.025-0.126

[1]Arithmetic mean. Values below the detection limit (0.01µg/l) are set to have concetration of half of detection limit.

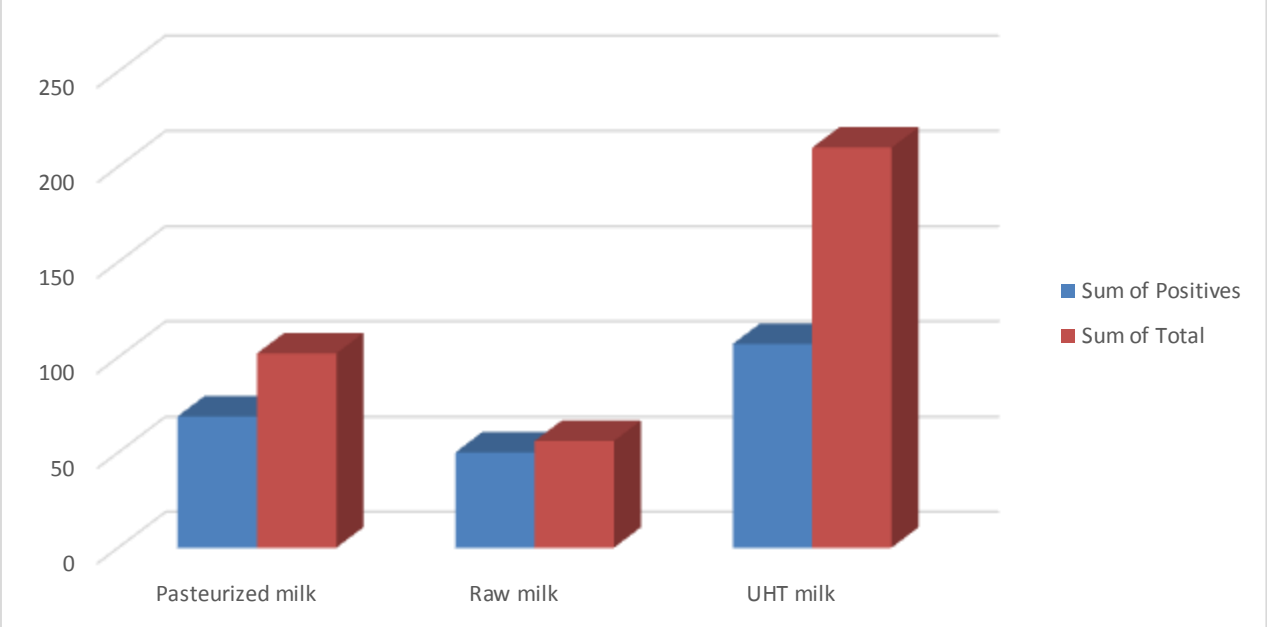


Chart 1. Occurrence of aflatoxin M1 (AFM1) in raw milk, pasteurized milk and UHT milk

The incidence of contamination was the highest in raw milk which is in compliance with the similar study where Vuković *et al.* (2013) reported that 85.6% samples of raw milk were contaminated by AFM1. Also, the highest percentage of the samples which exceeded concentration of 0.05 μg/kg was detected in raw milk samples (30.35%), followed by pasteurized milk (25.44%) and finally UHT milk (5.24%). This ratio between raw and heat treated milk samples is in accordance with results reported by Tomašević et al. (2015), where 56.3% of raw milk and 32.6% of heat-treated milk samples exceeded concentration of 0.05 μg/kg. Further, the maximum contamination level of aflatoxin M1 (2.10μg/l) in our study was found in raw milk. The obtained result is comparable to those performed in some other studies which have indicated high levels of AFM1 in raw milk samples. Jajić *et al.* (2018) reported that maximum concentration of AFM1 was in a raw milk samples, as well.

In addition, 4 samples of raw milk (7.14%) exceeded the maximum limit of 0.25μg/l set by Serbian regulative ("Official Gazette RS no. 81/2019"). Milićević *et al.* (2017) found that the AFM1 concentrations of 9% of raw milk were higher than Serbian maximum limit of 0.25μg/l, too.

Conclusion

The presence of AFM1 was detected in 226 out of 368 analyzed samples of raw milk, pasteurized milk and UHT milk. The highest percentage of contaminated samples was detected in raw milk (89.28%). The maximum contamination level of AFM1 (2.1μg/l) was found in raw milk, as well. Four samples were not in compliance with Serbian regulative ("Official Gazette RS no. 81/2019"), which is 7.14% of all analyzed samples of raw milk. In 14.67% of samples, maximum EU level for AFM1 in milk (0.05 μg/kg) was exceeded. Obtained results suggest still high contamination frequency of AFM1 in examined samples, especially, in samples of raw milk. Although, the level of contamination is significantly lower, percentage of contaminated samples of pasteurized milk (67.65%) and UHT milk (50.95%) is not negligible. Considering that aflatoxins are highly toxic and since milk represents one of the main foodstuffs in human diet in Serbia, there is a concern regarding human exposure to AFM1. Although, high contamination levels seldom occur in commercial milk, there is a need for permanent control and continuous monitoring over milk in order to avoid health risks related to presence of AFM1.

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Pravilnik o maksimalno dozvoljenim količinama ostataka sredstava za zaštitu bilja u hrani i hrani za životinje i o hrani i hrani za životinje za koju se utvrđuju maksimalno dozvoljene količine ostataka sredstava za zaštitu bilja („Sl. Glasnik RS", br. 81/2019) (Sr) Rule book on the maximum allowable amount of residues of plant protection products in food and feedstuff and on food and feedstuff for which shall be determined the maximum allowable amount of residues of plant protection products („Official Gazette of the RS", No. 81/2019) (EN).

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RISK OF INTRODUCTION OF QUARANTINE ORGANISMS: CASE *CLAVIBACTER MICHIGANENSIS* SUBSP. *SEPEDONICUS*

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Abstract

Transmission of plant pathogens and pests across country borders has raised an alert in the EU. Some of them having a severe economic impact, leading for taking precautionary measures such as quarantine of plant materials transported across borders. *Clavibacter michiganensis* subsp. *sepedonicus* (*Cms*) is listed as a quarantine organism in EU. This bacterium causes potato ring rot disease and presents a worldwide threat due to crop losses during vegetation and in storage. Potato tubers from import in Serbia were tested for its presence in accordance with the official EU Council Directive. From the stolon end of 200 tubers (consist one sample), the small core of tissue containing vascular tissue was removed and the heel ends were taken and crashed in sterile phosphate buffer, then centrifuged. Re-suspended pellet was used for immunofluorescence (IF, Loewe Biochemica GmbH), isolation of bacteria and DNA extraction for Polymerase Chain Reaction (PCR) performed with a pathogen-specific primer set PSA-1/PSA-R. As a positive control *Cms* reference strain CFBP 3561 was used. Two samples of ware potato originated from Belarus and Russian Federation in 2019 gave positive results for the presence of *Cms*. Visible internal symptom was observed on several tubers, in form of the vascular ring of tuber when they cut transversely. Bacterial ooze stream when tubers squeezed. The isolation of bacteria was performed from the ooze on Nutrient dextrose agar. Creamy-white, smooth colonies were formed after 3-5 days of incubation at 22 ºC. Restriction fragment length polymorphism (RFLP) of PCR products performed with PSA-1/PSA-R primer pair (502 bp) with enzyme *Bgl*II (fragments 282 and 220 bp in size) confirmed that the isolates belong to *Cms*. Pathogenicity was confirmed on aubergine seedlings, showing typical wilting obtained within 15-20 days after inoculation. Serbia is still free area from *Cms* pathogen due to rejection of contaminated potatoes from import.

Keywords: *health status, plant, disease, bacteria.*

Introduction

Plant quarantine represents a system of regulations established to protect agriculture production from losses caused by harmful organisms. It controls the introduction of harmful insects, mites, nematodes, gastropods, bacteria, phytoplasmas, fungi, viruses and virus-like organisms, and plants into free areas and as such is the best and most effective preventive measure for spreading of quarantine organisms. In the European Union (EU), phytosanitary measures are specified by EC Council Directive 2000/29/EC (EC, 2000), and 300 pests that are marked as quarantine are subjected to quarantine requirements (Schrader and Unger, 2003). There are two lists of quarantine pests recommended by EPPO (European and Mediterranean Plant Protection Organization), A1 list that includes pests absent from the EU, and A2 list with pests that are not widespread in the EU. According to EU standards, Serbian Ministry for Agriculture, Forestry and Water Management through the Plant Protection Directorate proposes Regulations for each harmful organism.

Clavibacter michiganensis subsp. *sepedonicus* Davis. *et al.* (*Cms*) causal agent of potato ring rot is listed in the European Plant Health Directive and may be very destructive to the potato crop. Since it is highly contagious and persistent in plant and soil debris, this bacterium is submitted to strict regulation to avoid its dissemination by plant transport. *Cms* represents a worldwide threat to potato growing and its industry due to crop losses. Losses are direct, during growth and in storage, and indirect, reflected through rejection of infected seed lots and the cost for the control measures, and by loss of export markets or difficulties in opening new markets (Van der Wolf *et al.*, 2005a; Van der Wolf *et al.*, 2005b; Węgierek-Maciejewska *et al.*, 2019; Charkowski *et al.*, 2020). Direct yield losses of over 50% have been estimated from field trials in Norway and the USA (Elphinstone, 2011). In Europe, the economic impact caused by *Cms* is estimated at 15 million Eur per year (Van der Wolf *et al.*, 2005a). Bacterium occurs in cool regions of America, Canada, China, Northern Europe and Russia (Van der Wolf *et al.*, 2005a). According to Charkowski *et al.* (2020) strict regulation in Europe reduced findings in annual surveys, with only occasional findings in some countries (Bulgaria, Czech Republic, Estonia, Finland, Germany, Greece, Hungary, Latvia, Lithuania, Netherlands, Norway, Slovakia, Sweden, Turkey). Isolated former outbreaks have been declared eradicated in Austria, Belgium, Cyprus, Denmark, France, Spain, and the UK (England and Wales) (Charkowski *et al.*, 2020).

The main symptoms caused by *Cms* are systemic infection of vascular tissue, interveinal chlorosis, and wilting/epinasty at leaf margins (Sadunishvili *et al.*, 2020; Charkowski *et al.*, 2020). The disease is difficult to detect in the field because the symptoms develop slowly, and latent infections are frequent. On tubers, *Cms* causes well-named ring rot of potato. As the infection usually starts on a tuber via infection in stolon, the infected vascular tissue of the tuber becomes yellowish and cheesy in texture due to bacterial oozing (Bragard *et al.*, 2019). As rot progresses, tuber surface cracks and dark blotches may become visible immediately beneath the periderm (Bragard *et al.*, 2019). The main sources of *Cms* transmission are infected seed potatoes (long distance) or contaminated equipment (Mansfeld-Giese, 1997; Van der Wolf *et al.*, 2005a). Seed damaged by cutting or by equipment can rise up a percentage of the infection to 80% (Van der Wolf *et al.*, 2005a). Bacteria can persist longer than 2 years on surfaces of different materials (iron, wood, rubber, and plastic). Possibility of surviving increases by a low relative humidity of 10%, and a temperature below 10 °C (Van der Wolf *et al.*, 2005a). *Cms* is able to survive in the postharvest debris of crops in the soil, and/or on alternative hosts (weeds) (Franc, 1999; Van der Wolf *et al.*, 2005b).

Management of potato ring rot is relying on the production and distribution of seed potatoes that are free from infection, achieved through strict application of quarantine and seed certification regulations, which involve a zero tolerance for the disease during seed and import inspections (Charkowski *et al.* 2020). In Serbia *Cms* is on List IA part I (Sl.glasnik RS, 2015). Potato tubers from import are being tested for *Cms* presence in accordance with EU Council Directive 93/85/EEC (EU, 1993) and EPPO PM 7/59(1) (OEPP/EPPO, 2006) standards. Quarantine procedure is based on laboratory screening of latent infection of *Cms* in tubers. This work presents cases of potatoes infected with *Cms*, found in the Serbian borders in 2019.

Material and Methods

Extraction of bacteria from plant tissue

Samples consisted of 200 potato tubers were first washed with water to free them from the soil impurities, surface-sterilized with sodium hypochlorite solution, and exposed to visual observations. From the stolon end of tubers, the small core of vascular tissue was removed and the ends were taken and transferred to a disposable maceration bag (Bioreba) where they

were crashed in sufficient volume of 50 mM phosphate buffer (PB). Supernatants were decanted and centrifuged at 7,000 *g* for 15 min, afterwards the pellet was re-suspended in 1 mL of sterile 10 mM PB.

Immunofluorescence (IF)
Undiluted extract of the samples and their 1:10 and 1:100 dilutions in 10 mM PB were subjected to IF assay using the antiserum Loewe Biochemica GmbH. Windows of the immunofluorescence slides were spotted by 20 μL of samples extracts and their dilutions, dried and then fixed. The polyclonal antibody and anti-goat fluorescein isothiocyanate conjugate (FITC) were diluted in PB according to the manufacturer's recommendation. Reference strain *Cms* CFBP 3561 (=PD 406) originated from potato (France) served as a positive control. Samples with the presence of green fluorescing cells with typical *Cms* size and morphology observed under the fluorescence microscope were considered as positive.

Polymerase Chain Reaction (PCR)
Bacterial DNA was extracted directly from potato extracts (Pastrik and Rainey, 1999; Pastrik, 2000). PCR was performed with a pathogen-specific primer set PSA-1 (5'-CTCCTTGTGGGGTGGGAAAA-3′) and PSA-R (5'-TACTGAGATGTTTCACTTCCCC-3′) (Pastrik and Rainey, 1999). PCR thermal conditions were as follows: initial denaturation at 95 °C for 3 min, 10 reaction cycles of 95 °C for 1 min, 64 °C for 1 min, 72 °C for 1 min, then 25 reaction cycles of 95 °C for 30 s, 62 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 5 min. DNA of *Cms* reference strain CFBP 3561 was used as a positive control. The products were visualized on a 1% agarose gel, stained with ethidium bromide. Band presence on the gel was checked under UV light. Amplified PCR products 502 bp in size were considered as positive.

Isolation of bacteria
Tubers from the samples which gave two test positive reactions were cut transversely. Isolation of bacteria was performed directly from the bacterial ooze on Nutrient Dextrose Agar (NDA) and kept at 22 ± 1 °C for 3-5 days. Presumptive colonies that were formed after the incubation period and had the morphology as *Cms* reference strain CFBP 3561 were purified and maintained at - 20 °C in Luria Bertani (LB) broth containing 20% (*v/v*) of sterile glycerol.

Pathogenicity
Pathogenicity of the obtained isolates was tested on young aubergine seedlings, planted in a sterile substrate and grown under the controlled conditions (22-25 °C, natural light and regular watering). Inoculations were performed in the phase of 3rd true leaf stage, appr. 3 weeks after sowing. The bacterial suspension (10^7-10^8 CFU mL^{-1}) was infiltrated in petioles of the 3rd true leaf, with a hypodermic syringe. *Cms* reference strain CFBP 3561 served as a positive control treatment and sterile distilled water as a negative. Inoculated plants were kept in plastic boxes and incubated for two weeks at 22 ± 2 °C and 70-80% humidity. Re-isolation from symptomatic plants was performed on NDA.

RFLP (Restriction Fragment Length Polymorphism)
For confirmation of isolates and re-isolates identity, RFLP analysis was used with PCR products obtained with PSA-1/PSA-R primer pair and incubation with enzyme *Bgl*II at 37 °C. Obtained amplicons were visualized under UV light on 1.5% agarose gel stained with ethidium bromide.

Results and Discussion

Two samples of ware potato originated from Belarus and Russian Federation in 2019 gave positive results for the presence of ring rot pathogen *Cms* using two diagnostic tests (i) IF test, after showing typical green fluorescing cells (Figure 2), and (ii) PCR with a pathogen-specific primer set PSA-1/PSA-R, after amplifying 502 bp size products. Although the IF test is accessible for detection of *Cms* (Dinesen and De Boer, 1995), non-specific reactions may occur (Van der Wolf *et al.*, 2005a). As a consequence, other tests, such as molecular PCR tests, should be used to verify positive results of IF. Although several PCR assays for *Cms* detection were developed, the most appropriate assays, validated based on specificity and sensitivity, are those given by Pastrik (2000) (Van der Wolf *et al.*, 2005a). According to EPPO distribution maps, *Cms* is present in Russia and Belarus (EPPO, 2020). In Russia, during the period 2015-2018, 39 samples were characterized as *Cms* positive and appeared in five Russian regions (Tver', Irkutsk, Kostroma, Leningrad, and Moscow) (Malko *et al.*, 2019). *Cms* belongs to the list of regulated nonquarantine pathogens in Russia, and after *Phytophthora infestans* it appeared to be most frequent with presence of 29.5% in total surveyed samples (Malko *et al.*, 2019).

A visible internal symptom was observed on a few tubers in two samples, in the form of a vascular ring of tuber when they were cut transversely. Bacterial ooze was expressed after a few seconds, when tuber was squeezed (Figure 1). This is in accordance with the symptom described in the literature (Charkowski *et al.*, 2020; Sadunishvili *et al.*, 2020). From bacterial ooze, white-creamy, smooth colonies were formed 3-5 days after isolation on the NDA (Figure 2). A total of ten representative isolates were subjected (five from each positive sample) to pathogenicity and further identification. In general, isolation of *Cms* from plant tissue is very difficult because it's slowly grown, and it is often overgrown on agar media by saprophytes (De la Cruz *et al.*, 1992; Dinesen and De Boer, 1995). Semi-selective media MTNA (Jansing and Rudolph, 1998) and NCP 88 (de la Cruz *et al.*, 1992) are appeared to be well suited for reliable *Cms* isolation procedure.



Figure 1. Potato ring rot

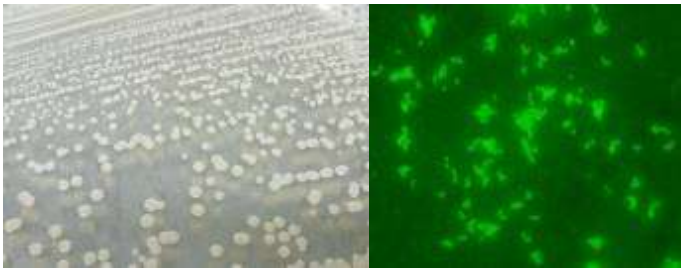


Figure 2. Bacterial colonies of *Cms* on NDA (left) and cells under the fluorescence microscope (right)

Typical wilting was developed on inoculated young aubergine seedlings within 15-20 days for all tested isolates and *Cms* reference strain CFBP 3561. Plants inoculated with sterile distilled water were symptomless. Re-isolation from aubergine plants confirmed the fulfilment of Koch's postulates.

In RFLP analysis, amplifyed products using PSA-1/PSA-R primer pair (502 bp in size) digested with *Bgl*II endonuclease gave the unique, characteristic RFLP pattern (282 and 220 bp in size) for all isolates and reisolates and the *Cms* reference strain CFBP 3561.

Based on the fact that this pathogen occurred in some southern countries, such as Spain, Cyprus, Greece, or neighbouring Bulgaria and Hungary (Charkowski *et al.*, 2020), *Cms* could be able to survive in Serbian environmental conditions. Therefore, strong standards for potato

tuber testing from import and intensive monitoring conducted for potato grown in Serbia is required.

Conclusions

Continuous monitoring of the *Cms* bacterium and rejecting of contaminated potatoes to be imported from the borders in Serbia by the authorities of the Ministry of Agriculture, Forestry and Water Management, gave the results that Serbia is still free area from the ring rot pathogen.

Acknowledgment

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OCCURENCE OF DEOXYNIVALENOL IN WHEAT BRAN IN SERBIA DURING 2019-2020

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Abstract

Animal welfare continues to be an important issue in the whole world. Therefore the production of safe feed remains one of the most important tasks because the consumption of contaminated feeds by livestock has been associated with a variety of adverse health effects including feed refusal, reduced weight gain, diarrhoea and emesis. Wheat bran, a by-product of the dry milling of common wheat (*Triticum aestivum* L.) into flour, is one of the major agro-industrial by-products used in animal feeding. In addition, wheat bran can also been used in human diet as a source of fiber in the preparation of high-fiber pasta, noodles etc. Deoxynivalenol (DON) is one of several mycotoxins produced by certain *Fusarium* species that frequently infect wheat, corn, rice, oats, barley and other grains in the field or during storage. In this study, a total of 72 samples of wheat bran were collected from several producers from Serbia in the period 2019-2020. The samples were analysed for deoxynivalenol (DON) content by using an enzyme-linked immunosorbent assay Ridascreen® Fast DON SC. The occurrence of DON in the tested samples was 100%, with average concentration 2.79 mg kg^{-1} and maximum DON content of 9.12 mg kg^{-1}. These results suggest very high level of DON concentration in all tested samples. The limit of 8.00 mg kg^{-1} imposed by Serbian legislation for DON content in feed was exceeded in two studied samples. However, only fourteen of them (19%) would be suitable for human consumption, due to a much lower allowed limit (0.75 mg kg^{-1}), which raises a risk for consumers.

Key words: *Deoxynivalenol, wheat bran, ELISA.*

Introduction

Wheat and wheat-based products are considered to be staple food for the majority of the world population (Škrbić et al., 2012). Although very rich in nutrients and popular among nutritionists who suggest a healthy diet, wheat bran is one of the major agro-industrial by-products used in animal feeding. Wheat bran, a by-product of the dry milling of common wheat (*Triticum aestivum* L.) into flour and it consists of the outer layers (cuticle, pericarp and seedcoat) combined with small amounts of starchy endosperm of the wheat kernel (www.feedipedia.org).

Animal welfare continues to be an important issue in the whole world. Therefore the production of safe feed remains one of the most important tasks because the consumption of contaminated feeds by livestock has been associated with a variety of adverse health effects including feed refusal (mainly by swine), reduced weight gain, diarrhea and emesis (Krska et al., 2001; Kuiper-Goodman, 2002).

Unfortunately, wheat like many other cereals is susceptible to fungal attack, therefore to possible mycotoxin contamination. Each year a large number of crops are affected by fungal invasion. Most of the infection is set in outer layers of the seed, which makes a wheat bran highly contaminated product. The most important agriculturally toxigenic fungi occurring in the moderate climatic zones of North America and Europe are *Fusarium* fungi (Kos et

al.2003). According to the literature, DON is the main *Fusarium* toxic secondary metabolite and its occurrence is also considered to be an indicator of the possible presence of other, more toxic, trichothecenes (Jajic et al., 2008). Although DON is among the least toxic of the trichothecenes, it is the most frequently detected one throughout the world (Lombaert, 2002). According to Serbian legislation maximum permitted level of DON in feed with a moisture content of 12 % in cereals and cereal products is 8000 μg kg^{-1} (Službeni glasnik RS, 27/2014). Significantly lower maximum permitted level is set for humans, 750 μg kg^{-1} (Službeni glasnik RS, 81/2019).

Although, occurrence and prevention of DON have been intensively studied, there are only a few of studies conducted in Serbia on retention of DON after harvest and during processing. The study on retention of DON during primary processing (milling) is important for the risk assessment and management for majority of world population (Kushiro, 2008). The objective of this study was the examination and determination of the presence of DON in wheat bran collected from Serbian producers in order to raise the awareness among consumer population.

Materials and Methods

Reagents and chemicals

RIDASCREEN FAST DON SC (R-Biopharm), a competitive enzyme immunoassay for quantitative analysis of DON in cereals, malt and feed was used according to manufacturer's instruction (RIDASCREEN FAST DON SC Art.No.:R5905). Distilled water was used for the extraction.

Collection of samples

From June 2019 until May 2020, 72 samples of wheat bran were collected from 5 producers from Autonomous Province of Vojvodina, as a part of the food safety control. Before analysis, the samples were stored at 20-25 °C and protected from light.

Sample preparation

All samples were thoroughly homogenized. Namely, 5 g of each sample of white bread and wholemeal bread was extracted by shaking with 100 ml of distilled water manually for 5 minutes. After shaking sample extracts were filtered through Whatman No.1 filter. 50 μL of the filtrate was used for further analysis according to RIDASCREEN FAST DON SC manual.

Instrumental conditions

The measurement is made photometrically at 450 nm. The absorbance is inversely proportional to the DON concentration in the sample. Multiskan FC microplate reader with absorbance range 0 - 6.000 A was used. Normal reading mode was used with reading speed t = 13 s. Using method was validated (LoD = 100 μg/kg, Recovery = 92%).

Results and Discussion

Out of the 72 samples analyzed, 100 % were contaminated with DON (Table 1). This level of occurrence of contamination is not surprising. In 2014 and 2017, 100 % of the samples were contaminated as well (Jaukovic et al., 2015) (Jaukovic et al., 2017). The obtained results are in compliance with the conclusions of the study conducted by Abbas et al. (1985) which shows that the distribution of DON is not uniform in the milling fractions and proves that the highest concentration of DON is always in bran. Trigo-Stockli et al. (1996) reported in the similar study that DON levels were the highest in the bran (3.4 mg/kg) as well. This could be due to the fact that after milling most of the concentration remains in outer layers (Tanaka et

al. 1986). However, the level of average DON concentration is significantly higher than in previous years (Table 1).

Last time this high concentrations were detected was in 2010 (Jajić et al., 2014).Among the 128 analyzed wheat samples from 2010, 100 (78.1%) samples were contaminated with DON. DON concentrations in positive samples ranged from 64 to 4808 µg/kg, with mean level of 779 µ/kg. In 16 of the examined wheat samples, the content of DON was above the maximum tolerable level (1250 µg/kg) adopted by the mentioned regulations.

Variations in DON concentration levels could occur due to a variety of factors such as wheat cultivars, the use of Good Agricultural Practices (GAPs), mold strain types, temperature, water activity, nutrient availability, and chemical agents applied (Bianchini et al., 2015). Jajić et al. (2014) pointed out that weather conditions during the plant growth, in particular in the flowering stage, have a major influence on the production of DON. According to the literature data, the optimal temperature for F. graminearum growth is 25 °C, at a water activity above 0.88. In addition, it is known that the incidence of FHB is strongly associated with moisture at the time of flowering (anthesis) and the timing of rainfall, rather than its amount (JECFA, 2001).Analysing samples from the 2010 harvest Jajić et al. (2014) definitely confirmed that the frequency of contamination is highly dependent on weather conditions. High humidity during May and June of 2010 contributed to the development of Fusarium already in the field, and thus the production of DON.

Table 1. Occurrence of DON in wheat bran in Serbia (2013-2020)

Year	No. of samples	No. of positive samples (%)	Average ± SD (µg kg^{-1})	Range (µg kg^{-1})
2013	14	5 (36)	93 ± 18.6	105-125
2014	28	28(100)	652 ± 130.4	343-1250
2015	12	10(83)	458 ± 91.6	120-1340
2016	15	15(100)	1074 ± 214.8	161-2790
2017	14	13(93)	831 ± 166.4	164-1950
2018	12	11(92)	586 ± 117.2	126-1740
2019	16	16(100)	2743 ± 548.6	378-9120
2020	56	56(100)	3068 ± 613.6	745-8510

*Source: Official control by Jugoinspekt Beograd

Conclusion

By analyzing the DON content in samples of wheat bran produced in Serbia in 2019 and 2020, it was found that the samples produced after the harvest in 2019 were 100% contaminated. Also, the average level of contamination in 2019 and 2020 is significantly higher than in previous years. Only 19% of the analysed samples would be suitable for human consumption and two of them even exceed the limits set for animal feed. This proves that natural occurrence of DON is strongly associated with moisture at the time of flowering (anthesis) and the timing of rainfall, rather than its amount. The obtained results for wheat bran confirmed that wheat should be continuously controlled to protect the population against the risk of mycotoxin contamination These results suggest a high percentage of contaminated samples, which raises a risk for consumers. A provisional tolerable daily intake (TDI) for DON was set in 2002 by the Scientific Committee for Food (SCF) at 1 µg/kg body weight (b.w.) per day.

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THE CHANGES OF INSECTICIDES DURING FERMENTATION PROCESSES

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Abstract

Pesticide use is incontrovertible part of agricultural production, but their residues remain on food commodities during post-harvest period causes serious health and trade problems. Scientific data revealed that some food handling, preparation and processing methods affected the amounts of pesticide residues. Fermentation is a microbiological food preservation process catalysed by the enzymes of yeasts, bacteria, and moulds, that transforms carbohydrates into simpler components such as alcohols, acids, and gases. Changes of pesticide levels and transformation to other degradation products during fermentation could be a result of chemical or biological degradation. Biodegradation by microorganisms is an important way for the breakdown of many pesticides. Microorganisms can metabolize some pesticides as carbon and energy sources and can also break them down with their extracellular enzymes. These microbial mechanisms are affected by factors such as pH, moisture, temperature and light. pH degrees lower than 4 are desirable for the microbiological safety of the many fermented products. Experiments showed that some lactic acid bacteria and yeasts were able to degrade chlorpyriphos and deltamethrin (invitro) by their esterase enzymes and could utilize them as carbon and energy sources. It was also observed that levels of dimethoate, deltamethrin, imidacloprid, malathion, chlorpyrifos-methyl and lambda-cyhalothrin were affected by the pH values and microbial diversity of the different fermentation conditions. Declines in microbial growth and pesticide degradation rates were detected at lower pH (≤4) values.

Keywords: *biodegradation, bacteria, yeasts, food safety, residue.*

Introduction

The pesticides are the most common additives for controlling the pests in agricultural production because of their quick and powerful effects (Simon, 2014). However, the presence of insecticide residues on fresh agricultural products and/or processed foods, causes crucial health problems on humans (González-Rodríguez *et al.*, 2011). Particularly, some chemical groups, namely, coppers, organophosphates, synthetic pyrethroids, neonicotonids, avermectins and insect growth regulators (IGRs) have been extensively used to control the plant pests. As an example for Turkey, chlorpyrifos, dimethoate, deltamethrin, lambda-cyhalothrin, beta-cyfluthrin, acetamiprid, imidacloprid, thiamethoxam, thiacloprid, emamectin-benzoate, pyriproxyfen are the most preferred insecticides against the plant pests (Kumral *et al.*, 2017). The use of 204 pesticides was restricted by The Turkish Ministry of Agriculture and Forestry due to their adverse effects on humans (Anonymous, 2020). For the proper use of pesticides, great attention must be paid to pre-harvest intervals (PHI) and good agricultural practices (GAP) (Cho *et al.*, 2009). Half-life of pesticides show diverse variances under natural conditions, depending on some factors such as hydrolysis, oxidation-reduction potential, photolysis, pH and temperature (Roberts *et al.*, 1999; Simon, 2014). If no attention is paid to PHI, the pesticide residues on foods remain above the recommended Maximum Residue Limits (MRLs) (EU Pesticides Database 2020).

To prevent spoilage of the fresh foods, di□erent food processing and preservation techniques are used. These techniques can be listed as cold storage, cooking, drying, smoking, fermentation and etc. The effect of processing and preservation techniques on the pesticide residue levels is in the focus of food safety researchers. The existence of pesticides in food commodities highly depends on the type of processing. The objective of this study is to discuss the effect of fermentation and related factors on the degradation of insecticides.

Microbial degradation during food fermentations

Fermentation is a microbiological process using enzymes in order to transform carbohydrates and proteins into simpler components. The microorganisms involved in the fermentation of food are commonly yeasts, bacteria, and moulds. The effect of fermentation on pesticide levels of foods have been reported in a varied extent (Bajwa and Sandhu, 2014; Regueiro *et al.*, 2015). Previous studies reported that some lactic acid bacteria (LAB) and yeasts can metabolize pesticides by their esterase enzymes and/or using the insecticides as carbon and energy sources (Regueiro *et al.*, 2015; Choi *et al.*, 2004; Islam *et al.*, 2010; Kumral *et al.*, 2020a; Zao and Wang, 2012; Dordevic *et al.*, 2013). Except for microbial metabolism, the biological degradation of the pesticides is dependent on some environmental parameters such as temperature, pH, moisture, and light, and their chemical features like volatility, insolubility in water, and adsorption ability to matrix compounds (Regueiro *et al.*, 2015).

During fermentation of some fresh vegetables or fruits (e.g. sauerkraut, natural black olives, kimchi), salt addition accelerates diffusion of fermentable substrates from plant cells and this liquid serves as growth medium for microbial flora. To inhibit spoilage microorganisms, low pH and anaerobic conditions must be provided (Steinkraus, 1992). The spontaneous fermentation via naturally occurring LAB namely, *Leuconostoc mesenteroides; Leuconostoc citreum, Leuconostoc argentinum, Leuconostoc fallax, Lactobacillus plantarum*; *Lactobacillus brevis, Lactobacillus paraplantarum, Lactobacillus coryniformis, Pediococcus pentosaceus,* present on fresh foods can decrease pH level rapidly (Harris *et al.*, 1992; Plengvidhya *et al.*, 2007; Müller *et al.*, 2018). Although enterobacteria, acetic acid bacteria, yeasts and moulds are seen at the beginning of the fermentation, their growth are stopped with increasing rates of lactic acid, acetic acid and carbon dioxide (Müller *et al.*, 2018). After that the dominant flora is LAB. Numerous researchers previously reported that some strains of LAB species belong to *Lactobacillus* and *Leuconostoc* genera can metabolize insecticides by esterase enzymes and/or by using them as carbon and energy sources (Cho *et al.*, 2009; Islam *et al.*, 2010; Zhao and Wang, 2012; Dordevic *et al.*, 2013; Kumral and Kumral, 2013; Kumral *et al.*, 2020a). LAB have gained much interest for their health benefits and are widely used as probiotics and starter cultures for fermented products because of their generally recognized as safe status (Maragkoudakis *et al.*, 2006). Previous studies showed that some LAB including *Lactobacillus* spp. are involved in the degradation of insecticides and may have the potential to be used for the decontamination of foodstuffs. Additionally, it is also showed that, the concentration of some organophosphate insecticides declined during the fermentation of kimchi, natural black olives, sauerkraut, wheat dough and Chinese cabbage (Regueiro *et al.*, 2015; Zhou and Zhao, 2015a; Kumral *et al.*, 2020b). The varied reduction rates (10-60%) in degradation of some insecticides (chlorpyrifos-ethyl, chlorpyrifos-methyl, malathion, dichlorvos) with effect of LAB during sauerkraut, kimchi and Chinese cabbage fermentations were reported by some authors (Cho *et al.*, 2009; Zhou and Zhao, 2015a). Kumral et al. (2020b) showed that 53−61% of deltamethrin, 66−68% of dimethoate, and 42−50% of imidacloprid were removed at the end of fermentation in natural black olives both with and without *L. plantarum* inoculation. Similarly, some insecticides were degraded during the fermentations of wine (Cabras and Angioni, 2000). Additionally, several studies showed that the ability of some yeasts species (*Saccharomyces cerevisiae, Candida boidinii*) to degrade

pyrethroid and OP insecticides (Fatichentietal *et al.*, 1984; Aislabie and Lloyd-Jones, 1995; Cabras *et al.*, 1995a; 1995b; 1995c; Regueiro *et al.*, 2015; Dordevic *et al.*, 2013; Kumral *et al.*, 2020c). A study on bread-making process demonstrated considerable loss of pesticides (47–89%). Similar results for the fermentation processes of animal products such as yogurt and skimmed milk are also reported (Zao and Wang, 2012; Zhou and Zhao, 2015b).

Effects of pH on the degradation of pesticides

The critical environmental factor is pH on the microbial stability of a fermented product. During lactic acid fermentations, the pH levels are generally decreased below 4 with the activities of LAB (Müller *et al.*, 2018). The acid-tolerant LAB (*L. plantarum*) produces lactic acid and declines the pH nearly 3.5 in plant based fermentations. The existence of the LAB species is a guarantee to provide high acidity degrees for the safety of the fermentation (Müller *et al.*, 2018). On the other hand, pH level of fermenting product significantly affects the degradation of the insecticides. Low pH conditions cause the deceleration of the degradation of many insecticides. The hydrolysis of organophosphate insecticides (OPs) was found more rapid under alkaline conditions (Roberts et al. 1999; El Beit *et al.* 1978; Lee and Lee 1997). For example, the half-life (DT_{50}) of some OPs (malathion, chlorpyrifos-ethyl, chlorpyrifos-methyl, dimethoate) is much longer in acidic pH levels (107, 73, 27 and 158 days, respectively) compared with alkali pH conditions (0.5, 23, 13 and 5 days, respectively) (PPDB database, 2020). In accordance with these data, several researches showed lower degradation of OPs in fermented cabbage samples (El Beit *et al.* 1978; Lee and Lee 1997; Roberts *et al.* 1999).

Effects of Temperature

Insecticides can be degraded faster at higher temperatures than lower ones (Simon, 2014). Castillo and Torstensson (2007) reported higher pesticide dissipation rates in 20 °C than in 2 and 10 °C. Fermentation takes place at different temperatures in different products (natural black olives at 10-15ºC, sauerkraut at 22-25ºC, yogurt 42ºC, wine at 20 ºC) of animal and plant origin (Kumral *et al.*, 2009; Bo *et al.*, 2011; Müller *et al.*, 2018; Cabras *et al.*, 1995c; Zao and Wang, 2012).

Effects of Photolysis

Photolysis causes degradation of molecules to smaller units through the absorption of sunlight. The ultraviolet (UV) region of sunlight can also destruct insecticides after application to plants, depending on exposure time, intensity and wavelength of light, chemical structure of pesticides and temperature (Simon, 2014). The advantage of photolytic pesticide degradation cannot always be taken since, almost all food fermentations performed in opaque containers under dark conditions.

Effects of Dehydration

Water solubility of pesticides is an important factor for reducing their residues on solid foods. Additionally, lower moisture contents in foods decrease degradation rate of the water soluble pesticides by bacteria during fermentation (Azizi, 2011). Although the fermentation impact on reduction of pesticide residues in vegetal products was reported several times, most of them are for liquid foods such as alcoholic beverages rather than solid foods (Cabras *et al.*, 1995a; 1995b; 1995c; Navarro *et al.*, 2007; Bo *et al.*, 2011; Regueiro *et al.*, 2015). The water contents of some vegetables (sauerkraut) or fruits (table olives) are decreased during fermentation or processing by the addition of salt (Anonymous, Müller *et al.*, 2008). Adding salt in the fermentation medium of the food increases the pesticide concentration on dry matter of food due to low solubility of some pesticides in water (Kumral *et al.*, 2020b).

Effects of pesticide structure

Pesticides are members of varied chemical groups. To date, more than 60 different chemical groups registered as insecticides (IRAC, 2020). The half-life of insecticides is very variable depending on their water solubilities, pH stabilities, volatilities, photo-degradation sensibilities, sizes and binding types of molecules (Simon, 2014; PPDB, 2020). Many insecticides have rigid stability under acidic conditions, but some compounds such as diazinon, thiacloprid, acetamiprid, imidacloprid, are more stable at alkali conditions (Simon, 2014). The degradation rates of insecticides belonging to different chemical groups display variations in the same fermentation conditions (Kumral *et al.*, 2020b). These differences could be attributed to the differences in their chemical nature (Zhou and Zhao 2015a; Bo *et al.* 2011).

Conclusions

Fermentation affects pesticide residue levels of final product in a varied extend. These effects are related with many factors and each fermentation process has its unique characteristics. Fermentation sometimes plays an important role for the decontamination of pesticide residues, but on the other hand contradictory effects are seen depending on the chemical structure of the pesticide and the conditions like low pH, low temperature, darkness and dehydration. So, to avoid from the adverse effects of pesticide residues, fermentation conditions must be carefully evaluated and PHI and GAP guidelines must be strictly followed.

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COMPARATIVE ANALYSIS OF INDIVIDUAL PHENOLIC COMPOUNDS IN COMMERCIAL TYPE STRAWBERRIES, RASPBERRIES AND BLACKBERRIES

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Abstract

Biologically active components such as polyphenols are becoming an area of interest in the research of many scientists. Furthermore, polyphenols have a very important role as functional foods in our nutrition having many effects on prevention and protection from several specific diseases i.e. certain polyphenols can stop several diseases' development via certain mechanisms. This research paper aims to identify and quantify individual polyphenolic components in the commercial type of strawberries, raspberries, and blackberries. In the study conducted in 2019, different commercial types of fruits were purchased in retail stores in Atakum, Samsun-Turkey. Analyses were performed in Amasya Central Research Laboratory. Identification and quantification of biologically active components, individual phenols, were carried out by the Ultra Fast Liquid Chromatograph system technique. Detection occurred at 280 nm and 520 nm. The levels of individual phenolic compounds were compared; Catechin 6,57 mg/100g was the dominant individual phenol in strawberries and caffeic acid was in the dominant individual phenol in blackberries 21mg/100gr and raspberries 29,19 mg/100g. The obtained results confirmed that the examined fruit species were rich in biologically active substances, polyphenols, and, as such were welcome on greengrocers shelves and recommended for daily consumption.

Keywords: *individual polyphenols, strawberries, raspberries, blackberries, functional food.*

Introduction

Fruits as strawberries, raspberries, and blackberries contain appreciable levels of polyphenols, vitamins, and minerals. Polyphenols are a large family of naturally occurring compounds in fruits, which are produced by plants. They are important as functional foods in our nutrition and play a valuable role in organoleptic properties of colour and taste in numerous food products.

Their chemical diversity is matched by their various role in the plant world, for example, phenolics can function as antibiotics, to prevent water loss, as natural pesticides, to signal substances for the establishment of symbiosis with rhizobia, as attractants for pollinators, to protect the plant from harmful ultraviolet solar radiation and as structural materials to give plants stability**. (**de la Rosa et al., 2010; Shahidi and Naczk, 2006**)**.

Phenolic compounds are known to have many biological activities such as antioxidant characters. Furthermore, there is epidemiological evidence indicating that the nutrition of diets rich in fruit is correlated with a lowered risk of a numerous chronic diseases, neurodegenerative diseases, and cancers. The average daily intake of dietary polyphenols is about 1 g per person. Phenolic compounds are secondary metabolites of plants and have in their structure minimum one aromatic ring with one or more hydroxyl groups attached. The main categories of phenolic compounds found in fruit are phenolic acids, flavonoids, tannins, and stilbenes (Güneş Bayir et al., 2019; Del Rio et al., 2010; Shahidi and Naczk, 2006;

Manganaris et al., 2013; de la Rosa et al,. 2010). The aim of this study was to identify and quantify individual phenolic compounds in commercial type strawberries, raspberries, and blackberries in Atakum, Samsun.

Materials and methods

Different commercial species of fruits (strawberries, raspberries, and blackberries) were purchased in retail stores in Atakum, Samsun-Turkey. The berry samples were treated with liquid nitrogen and stored at -80ºC in nylon boxes until analysis. Analysis were performed in 2019 in the Amasya Central Research Laboratory at the Amasya University in Turkey.
Extraction of selected fruit sample analysis of individual polyphenols was performed according to the method of WU.M.M. Et al. (2015), with some modification.
Analytical standards were: gallic acid, ellagic acid, p-coumaric acid, sinapic acid, ferulic acid, caffeic acid, neochlorogenic acid, procyanidin B1, procyanidin B2, catechin, epicatechin, quercetin 3-glucoside, quercitrin 3-ramnozid, quercetin dehydrate, kaempferol, keracyanin chloride, and rutin.
The chromatographic separation was performed using the Shimadzu Ultra Fast Liquid Chromatograph system (UFLC, Shimadzu Corporation, Kyoto Japan) equipped with a pump LC-20AD, diode array detector SPD-M20A, autosampler SIL-20AHT, room temperature.
Chromatographic separation was performed on a C18 Intersil ODS-3 column (GL Science Inc., Japan) analytical column (4.6x250mm, 5 μm). A gradient consisting of (A) 1% acetic acid and (C) acetonitrile was in chromatographic separation. Wavelength 280nm and 520nm.
The injected volume was 10 μL. The studied phenolic compounds were successfully separated in 65 min and the flow rate was 0.7 mL/min.

Table 1. Time-scheduled gradient elution program

	Time	Module	Action	Value
1	0.2	Pumps	C	10
2	28.00	Pumps	C	40
3	39.00	Pumps	C	60
4	50.00	Pumps	C	90
5	55.00	Pumps	C	10
6	65.00	Pumps	C	10
7	65.00	Controller	Stop	

Results and Discussion

Total phenolic compounds were obtained by the summing of individual phenolic compounds results. The highest content was found in raspberry samples 76.76 mg/100g, blackberries samples 39.47 mg/100g and the lowest in strawberry samples, 20.33 mg/100g.

Table 2. Content of individual phenolic compounds in strawberries, raspberries, and blackberries (mg/100g)

No.	Individual phenolic compounds	Strawberries	Raspberries	Blackberries
1.	Gallic acid	2.06	14.76	1.55
2.	Ellagic acid	0.14	0.06	ND
3.	p-Coumaric acid	ND	ND	TR
4.	Sinapic acid	ND	0.39	0.90
5.	Ferulic acid	ND	ND	ND
6.	Caffeic acid	2.78	29.19	21
7.	Neochlorogenic acid	2.02	2.38	4.06
8.	Procyanidin B_1	3.03	4.6	2.97
9.	Procyanidin B_2	3.44	2.42	2.05
10.	Catechin	6.57	5.68	3.63
11.	Epicatechin	ND	2.04	1.88
12.	Quercetin 3-glucoside	TR	ND	0.01
13.	Quercitrin 3-ramnozid	ND	ND	0.39
14.	Quercetin dehydrate	ND	ND	ND
15.	Kaempferol	ND	ND	ND
16.	Keracyanin chloride	0.29	15.02	1.03
17.	Rutin	ND	0.22	TR

*Source: Author s' elaboration based on the **obtained results**, ND not detected, TR traces detected.

The contents of individual phenolic compounds among the strawberries, raspberries, and blackberries examined varied considerably. The dominant individual phenolic compound in strawberries was catechin 6,57 mg/100g, in raspberries 29,19mg/100g and blackberries 21mg/100g caffeic acid was the dominant individual phenolic compound.

Gallic acid, caffeic acid, and neochlorogenic acid were detected in all samples whilst ferulic acid was not detected in any sample. Total phenolic acids were obtained by summing individual phenol acids. When comparing the samples, highest level of phenolic acid was found in raspberries 46,78 mg/100g.

From flavan-3-ols in this study detected procyanidin B1, procyanidin B2, catechin in all three samples of fruits, the exception was a strawberry in which epicatechin was not detected. Raspberries have the highest level of procyanidin B_1 4,6 mg/100g.

Quercetin 3-glucoside, quercitrin 3-ramnozid were detected in blackberries. The only anthocyanin that was analyzed in this study was keracyanin chloride and identified and quantified in all fruit samples and the greatest value detected in raspberry samples.

Conclusion

The highest phenolic content was found in raspberries 76,76 mg/100g and the lowest in strawberries 20,33 mg/100g.
Catechin 6,57 mg/100g was dominant individual phenolic compound in strawberries and caffeic acid was in the dominant phenolic compound in blackberries 21mg/100gr and in raspberries 29,19 mg/100g.
These fruit species are rich in phenolic compounds and thus recommended for every day consumption.

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BIOPOLYMER PRODUCTION FROM ISOLATED LACTIC ACID BACTERIA FROM IRANIAN KISHK

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Abstract

Exopolysaccharides are high molecular weight polymers composed of sugar subunits. Produced exopolysaccharides by lactic acid bacteria play a significant role in improvement the sensory and texture properties of fermented dairy products such as yogurt. Diversely, the probiotic function of these bacteria and the prebiotic properties of their produced biopolymers promote consumer health. For this purpose, a traditional dairy product known as "Kishk" was selected. 143 strains of lactic acid bacteria were isolated from Iranian Kishk in Khorasan Province and cultured in formulated MRS mediums with different sugars like glucose, fructose, sucrose and, lactose (40 g/L) and incubated in anaerobic conditions at 30 and 37 ° C for 48 hours. The microscopic features of the isolates were assessed and the production of exopolysaccharide in the culture medium was evaluated by disk and ruthenium red methods. The phenol-sulfuric and weight method were used to quantify exopolysaccharide production. The results showed the pH of Kishk samples ranged from 3.6 to 4.08 and the average of total mesophilic count and Lactic acid bacterial count of samples were 6.5 and 5.89 log CFU/g, respectively. Analysis of data exhibited 79 out of 143 lactic acid bacteria isolates were exopolysaccharide producer and 70% of them were cocci. The maximum and minimum production by weight method were 5.34 g/L and 0.002 g/L, respectively. The highest and the lowest amount of exopolysaccharide by phenol sulfuric method were measured 3.81 g/L and 0.0015 g/L, respectively. This study indicates the potential of exopolysaccharide production by Iranian native species from dairy products.

Keywords: *Exopolysaccharide, Lactic acid bacteria, Kishk*.

Introduction

The increased demand for natural polymers in various industrial applications during recent years has led to a renewed interest in exopolysaccharide (EPS) production by microorganisms. Many microorganisms have an ability to synthesize extracellular polysaccharides and excrete them out of cell (Suresh Kumar et al., 2007). New microbial polysaccharides might have innovative uses as gelling agent, emulsifier, stabilizer or texture enhancing agent (Sutherland, 2001).

Efforts have been made to use LAB as microbial cell factories for the production of industrially interesting metabolites either to be used as purified compounds or to be produced *in situ* in fermented foods (Boguta et al., 2014). As a traditional dairy product, Kishk is

mostly produced from sheep's milk. Firstly, raw milk is boiled and then cooled and inoculated with traditional yoghurt made earlier as starter culture. The butter is isolated from sour yoghurt by Mashk, which is made from hide (sheepskin) and is used for butter making. Then buttermilk is boiled and sieved by cloth bag. Finally the thick whitish semi-solid part of buttermilk, which is sieved, is shaped in form of conic or cubic balls and then sun-dried for 3–4 days (Iranmanesh et al., 2018). A wide variety of carbon sources, used to produce microbial exopolysaccharides, include sucrose, glucose, lactose, maltose, mannitol, sorbitol, whey, starch, sugar concentrates. The type of carbon source influences the yield of exopolysaccharide. The size of the exopolysaccharide may also vary with the carbon source (Suresh Kumar et al., 2007). Exopolysaccharide concentration is estimated as neutral carbohydrate content usually determined by the phenol sulfuric acid method or by weighting the polymer dry matter (Vaningelgem et al., 2004, Dubois et al., 1956).
This study demonstrates the effect of type of carbon source on exopolysaccharide yield in EPS producing lactic acid bacteria isolated from Iranian Kishk.

Materials and Methods

Chemical and Microbiological analyses:
The pH value of the Kishk samples was measured using a pH meter. The total number of mesophilic aerobic bacteria were enumerated on Nutrient Agar (NA) incubated at 35±2 °C for 24 h. MRS agar was used for counting Lactic acid bacteria (Kirdar and Advances, 2012).
Isolation of Lactic acid bacteria:
Kishk samples were taken from eight regions in Khorasan province according to Iranian National Standard No. 326. For the isolation of LAB strains from Kishk samples, 10 gr of samples were taken aseptically and transferred to 90 ml skim milk followed by the preparation of serial dilutions. Streak culture method on MRS (de Man, Rogosa and Sharpe) agar was done and the plates incubated at 30°C and 37°C for 24 h. Colonies with typical characteristics of lactic acid bacteria were selected from MRS and tested for Gram stain, cell morphology, Oxidase and catalase reaction (Ispirli and Dertli, 2017).
Screening of EPS producing isolates:
EPS synthesis was performed in MRS agar medium with disc and ruthenium red methods. In the disc method, MRS culture medium was formulated with 40% of 4 kinds of sugars (sucrose, fructose, lactose and glucose) instead of glucose in the main formula and the paper discs were inoculated with inoculum, incubated at 30 and 37 °C for 48 h (Paulo et al., 2012). In ruthenium red method, streak culture method was performed by overnight cultures on MRS-RR medium (0.08 g/l ruthenium red). After 48 h of incubation at 30 and 37°C, ruthenium red stains the bacterial cell wall, producing pink colonies for non-EPS producers and white colonies for producers(Hongpattarakere et al., 2012).
Isolation of EPS and determination of EPS:
For the isolation of EPS, all strains were grown in formulated MRS broth, inoculated at 1% (v/v) with an overnight culture then incubated at 30 and 37°C for 2 d anaerobically. Then centrifuged at 10,000 ×g for 10 min to remove the cells. Three volumes of 96% cold ethanol were added to the supernatant and stored overnight in 4 °C to precipitate. The culture was then centrifuged again (10,000 g for 10 minutes). After removing the supernatant, the precipitate was mixed with a sevag reagent (chloroform: n-butanol = 4: 1) and then centrifuged. The dialysis (cut-off 8000-12000Da) of the resulting supernatant with distilled water was performed at 4°C for 48 hours. The produced EPS was stored for 24 hours in an -80 °C freezer and then lyophilized. Determination of EPS was performed via phenol-sulfuric acid method using glucose standard as well as weight method by measuring the dry weight of EPS at 60 °C in an oven (Lin and Chien, 2007, Jeong et al., 2017, Dubois et al., 1956).

Statistical analysis:
Each test was performed in duplicate. Data from each test were subjected to SPSS (version 22.0.0.0) for analysis of variance. Duncan's multiple range test was used to determine any significant difference ($p < 0.05$) among treatments.

Results and Discussion

Chemical and Microbiological characteristic:
The results concerning chemical and microbiological properties (mean values and standard deviation) of the Kishk samples are shown in Table 1. The mean value of pH ranged from 3.62-4.08 in Kishk samples from different region of Khorasan province in Iran. Noori et al (2013) reported the pH of Kishk samples were in a range of 3.85-4.11 (Noori et al., 2013). According to Gadallah et al (2019) in Kishk samples pH ranged between 4.39–4.84 (Gadallah and Hassan, 2019). The average of total aerobic mesophilic bacteria and lactic acid bacteria were 6.5 and 5.89 log CFU/g in Kishk samples, respectively. The results of Kirdar et al (2012) showed the mean values of total bacterial count, *Lactobacillus* sp., *Lactococcus* sp. and *Enterobacteria* in Kishk samples were 8.24 ±0.95, 7.63±0.99, 7.52±0.96 and 2.62±1.13, respectively. These findings were higher than that of this study. A high number of TAMB can be explained by sufficient change in the environmental conditions which occur during Kishk storage and which allows for the growth of microorganisms (Kirdar and Advances, 2012). Total count and Lactic acid bacterial count of Kishk samples in different researches have been reported in a range of 3.46- 7.4 and 3.65- 4.89 log cfu/g, respectively (Tamime and Robinson, 2007). Total bacterial counts of different Kishk samples in work of Gadallah et al (2019) were ranged from 5.15 to 7.50 log cfu/g and Lactic acid bacterial counts were ranged from 6.04 to 7.88 log cfu/g (Gadallah and Hassan, 2019). There was a significant positive correlation between total aerobic mesophilic bacteria and lactic acid bacteria ($r=0.93$, $P\leq0.05$). These results were in agreement with the findings of Kirdar et al (2012) (Kirdar and Advances, 2012).

Table1- pH and microbiological properties of Kishk samples

Code of Samples	**pH** *	Total lactic acid bacteria* (TLAB)	Total aerobic mesophilic bacteria (TAMB)*
Q	$3.66\pm0.014^{c**}$	6.08 ± 0.38^{c}	7.02 ± 0.4^{b}
K	3.675 ± 0.007^{c}	5.45 ± 0.35^{e}	5.85 ± 0.27^{e}
T	4.04 ± 0.056^{a}	7.2 ± 0.41^{a}	7.8 ± 0.42^{a}
M	3.62 ± 0.028^{d}	5.36 ± 0.22^{f}	6.03 ± 0.32^{d}
S	3.85 ± 0.07^{b}	5.64 ± 0.41^{d}	5.8 ± 0.2^{e}
N	4 ± 0.035^{ab}	5.38 ± 0.3^{ef}	5.68 ± 0.41^{f}
R	3.85 ± 0.07^{b}	5.9 ± 0.3^{c}	6.75 ± 0.43^{c}
F	4.08 ± 0.042^{a}	6.14 ± 0.34^{b}	7.1 ± 0.35^{b}

*Values are reported as (mean ± sd)
**The letters in each column show statistical differences

Isolation of EPS producers and quantification of EPS:
The findings displayed among the 143 Gram-positive, non-spore forming, catalase and oxidase negative isolates, 79 isolates were detected putative EPS positive with both disk and ruthenium red methods. Amount of EPS was determined by phenol-sulfuric method and weight method. The results are presented in Table 2. These isolates had a mucoid character.

Ruas-Madiedo et al (2007) demonstrated 92% of their isolates were mucoid. It was in accordance with our results which states mucoid phenotype is dominant phenotype. According to the observation the maximum EPS production by weight method was, 5.34 g/L and minimum production 0.002 g/L, and the greatest amount of exopolysaccharide by phenol sulfuric method was 3.81 g/L and the lowest amount was 0.0015 g/L (Ruas-Madiedo et al., 2007). The amount of EPS production in different references is variant. *Streptococcus thermophilus* CC30 produce 1.95 g/L of EPS when grown in skim milk lactose medium at 30°C (Kanamarlapudi and Muddada, 2017). The EPS produced by the wild and mutant *L. delbrueckii* ranged from 5570.34 – 5910.62 mg/L (Adebayo-Tayo and Fashogbon, 2020). A high molecular weight EPS was recovered and purified to a yield of 2.8 ± 0.5 g/L from *Lb. plantarum* BR2 (Sasikumar et al., 2017). The highest EPS producing strains isolated from boza, yielding 2.39 ± 0.49 and 1.98 ± 0.23 g/L of EPS, respectively (Heperkan et al., 2014). *Lactobacillus kefiranofaciens* DN1 produced EPS, using glucose and lactose, and EPS yield rose to 2.2 g/L in modified MRS broth (60 g/L glucose) (Jeong et al., 2017).
The reported yields of HePS range from 50 to 350 mg/L for *Strep. thermophilus*, 60 to 150 mg/L for *Lb. delbrueckii* spp. *bulgaricus*, 25 to 600 mg/L for *Lc. lactis* spp. *cremoris*, and from 50 to 60 mg/L for *Lb. casei* (Ruas-Madiedo and De Los Reyes-Gavilán, 2005).

Table 2- EPS yield in different culture medium by two methods

code	Cell morphology	Growth temperature	Culture medium	Weight method EPS g/l	Phenol-sulfuric method
k65	bacilli	37	MRS-F	1.079545±0.08	0.771104±0.05
			MRS-L	0.2964±0.027	0.211745±0.01
			MRS-S	1.136364	0.811688
			MRS-G	0.550087±0.04	0.392919±0.02
R53	bacilli	37	MRS-F	0.596591±0.04	0.426136±0.02
			MRS-L	0.413043±0.025	0.295031±0.01
			MRS-S	0.744318±0.07	0.531656±0.05
			MRS-G	0.405327	0.289519
K63	cocci	37	MRS-F	1.022727	0.730519
			MRS-L	0.583004±0.013	0.416431±0.009
			MRS-S	0.960227±0.07	0.685877±0.05
			MRS-G	0.593515±0.02	0.423939±0.01
R118	cocci	37	MRS-F	1.681818±0.03	1.201299±0.02
			MRS-L	0.488142±0.019	0.348673±0.01
			MRS-S	1.477273	1.055195
			MRS-G	0.361899±0.02	0.258499±0.01
R105	cocci	37	MRS-F	1.727273±0.03	1.233766±0.02
			MRS-L	0.256917±0.027	0.183512±0.01
			MRS-S	0.880682±0.04	0.629058±0.02
			MRS-G	0.66879±0.036	0.477707±0.02
R116	cocci	37	MRS-F	0.909091	0.649351
			MRS-L	0.254941±0.02	0.182101±0.01
			MRS-S	1.028409±0.008	0.734578±0.005
			MRS-G	0.405327	0.289519
R50	cocci	37	MRS-F	0.715909±0.048	0.511364±0.03
			MRS-L	0.217391±0.027	0.15528±0.01

			MRS-S	0.823864±0.04	0.588474±0.02
			MRS-G	0.463231	0.330879
K67	bacilli	37	MRS-F	1.767045±0.08	1.262175±0.06
			MRS-L	0.466403±0.01	0.333145±0.007
			MRS-S	0.960227±0.07	0.685877±0.05
			MRS-G	0.961204±0.049	0.686575±0.03
S1	cocci	37	MRS-F	0.517045±0.07	0.369318±0.05
			MRS-L	0.221344±0.02	0.158103±0.01
			MRS-S	0.738636±0.08	0.527597±0.057
			MRS-G	0.605096±0.036	0.432211±0.02
K64	bacilli	37	MRS-F	0.852273±0.08	0.608766±0.057
			MRS-L	0.278656±0.002	0.19904±0.001
			MRS-S	0.960227±0.07	0.685877±0.05
			MRS-G	0.263463±0.03	0.188188±0.02
R114	cocci	37	MRS-F	3.75	2.678571
			MRS-L	1.047431±0.027	0.748165±0.01
			MRS-S	3.806818±0.08	2.719156±0.057
			MRS-G	1.8674±0.02	1.333857±0.01
K60	cocci	37	MRS-F	3.125±0.08	2.232143±0.05
			MRS-L	1.150198±0.005	0.82157±0.003
			MRS-S	2.596591±0.02	1.854708±0.01
			MRS-G	0.89751±0.04	0.641079±0.02
K72	cocci	37	MRS-F	1.028409±0.008	0.734578±0.005
			MRS-L	1.284585±0.027	0.917561±0.01
			MRS-S	2.329545±0.08	1.663961±0.057
			MRS-G	1.4273310.02	1.019522±0.02
K56	cocci	37	MRS-F	4.034091±0.08	2.881494±0.05
			MRS-L	1.343874	0.95991
			MRS-S	2.670455±0.08	1.907468±0.05
			MRS-G	1.476549±0.04	1.054678±0.02
K54	bacilli	37	MRS-F	3.357955±0.07	2.398539±0.05
			MRS-L	1.205534±0.02	0.861095±0.01
			MRS-S	2.5	1.785714
			MRS-G	1.395483±0.008	0.996774±0.005
T52	cocci	37	MRS-F	3.352273±0.08	2.394481±0.05
			MRS-L	1.12253±0.02	0.801807±0.01
			MRS-S	3.920455±0.08	2.800325±0.05
			MRS-G	1.160973±0.004	0.829266±0.003
K66	cocci	37	MRS-F	3.079545±0.01	2.199675±0.01
			MRS-L	1.007905±0.02	0.719932±0.01
			MRS-S	3.068182±0.16	2.191558±0.11
			MRS-G	1.357846±0.03	0.96989±0.02
K48	cocci	37	MRS-F	2.897727±0.08	2.069805±0.05
			MRS-L	1.067194	0.762281
			MRS-S	3.346591±0.07	2.390422±0.05

			MRS-G	1.375217±0.02	0.982298±0.01
R44	cocci	37	MRS-F	3.25±0.060	2.321429±0.04
			MRS-L	0.889328±0.02	0.635234±0.01
			MRS-S	2.244318±0.04	1.603084±0.02
			MRS-G	1.418645±0.04	1.013318±0.02
K55	cocci	37	MRS-F	2.732955±0.008	1.95211±0.005
			MRS-L	1.043478±0.02	0.745342±0.01
			MRS-S	3.636364	2.597403
			MRS-G	2.052693±0.04	1.466209±0.03
R34	cocci	37	MRS-F	0.454545	0.324675
			MRS-L	0.203557±0.008	0.145398±0.005
			MRS-S	0.170455±0.08	0.121753±0.05
			MRS-G	0.489288±0.03	0.349491±0.02
K61	cocci	37	MRS-F	0.170455±0.08	0.121753±0.05
			MRS-L	0.219368±0.03	0.156691±0.02
			MRS-S	0.170455±0.08	0.121753±0.06
			MRS-G	0.303995±0.02	0.21714±0.01
T69	cocci	30	MRS-F	0.494318±0.05	0.353084±0.04
			MRS-L	0.23913±0.002	0.170807±0.002
			MRS-S	0.738636±0.08	0.527597±0.05
			MRS-G	0.130284±0.02	0.09306±0.01
K62	cocci	37	MRS-F	0.607955±0.05	0.434253±0.04
			MRS-L	0.258893±0.03	0.184924±0.02
			MRS-S	0.829545±0.04	0.592532±0.03
			MRS-G	0.231616	0.16544
B4	bacilli	37	MRS-F	0.823864±0.04	0.588474±0.02
			MRS-L	0.108696±0.013	0.07764±0.01
			MRS-S	0.971591±0.07	0.693994±0.05
			MRS-G	0.416908±0.01	0.297791±0.01
S21	cocci	37	MRS-F	0.619318±0.07	0.44237±0.05
			MRS-L	0.357708±0.002	0.255505±0.001
			MRS-S	0.795455	0.568182
			MRS-G	0.550087±0.04	0.392919±0.02
T22	cocci	37	MRS-F	0.801136±0.008	0.57224±0.005
			MRS-L	0.332016±0.02	0.237154±0.01
			MRS-S	0.625±0.08	0.446429±0.05
			MRS-G	0.182397±0.01	0.130284±0.008
R30	bacilli	37	MRS-F	0.846591±0.07	0.604708±0.051
			MRS-L	0.296443±0.02	0.211745±0.01
			MRS-S	0.596591±0.04	0.426136±0.02
			MRS-G	0.422698±0.02	0.301927±0.01
S2	cocci	37	MRS-F	1.153409±0.02	0.823864±0.01
			MRS-L	0.332016±0.02	0.237154±0.01
			MRS-S	0.511364±0.08	0.36526±0.05
			MRS-G	0.356109±0.01	0.254363±0.008

R29	bacilli	37	MRS-F	0.625±0.08	0.446429±0.05
			MRS-L	0.20751±0.01	0.148221±0.009
			MRS-S	0.846591±0.07	0.604708±0.05
			MRS-G	0.535611±0.02	0.382579±0.01
T20	cocci	37	MRS-F	0.477273±0.03	0.340909±0.02
			MRS-L	0.256917±0.027	0.183512±0.01
			MRS-S	0.380682±0.05	0.271916±0.04
			MRS-G	0.179502±0.008	0.128216±0.005
T113	cocci	37	MRS-F	0.431818±0.03	0.308442±0.02
			MRS-L	0.217391±0.027	0.15528±0.01
			MRS-S	0.329545±0.01	0.23539±0.01
			MRS-G	0.057904	0.04136
T125	bacilli	37	MRS-F	0.596591±0.04	0.426136±0.02
			MRS-L	0.243083±0.008	0.173631±0.005
			MRS-S	0.857955±0.08	0.612825±0.06
			MRS-G	0.315576±0.036	0.225412±0.02
K68	bacilli	37	MRS-F	0.471591±0.02	0.336851±0.017
			MRS-L	0.288538±0.01	0.206098±0.01
			MRS-S	0.846591±0.07	0.604708±0.05
			MRS-G	0.495078±0.04	0.353627±0.03
R38	cocci	37	MRS-F	0.164773±0.07	0.117695±0.05
			MRS-L	0.209486±0.01	0.149633±0.01
			MRS-S	0.625±0.08	0.446429±0.05
			MRS-G	0.199768±0.036	0.142692±0.02
K75	bacilli	37	MRS-F	0.352273±0.01	0.251623±0.01
			MRS-L	0.209486±0.01	0.149633±0.01
			MRS-S	0.505682±0.07	0.361201±0.05
			MRS-G	0.37927±0.04	0.270907±0.03
K73	cocci	37	MRS-F	0.289773±0.07	0.206981±0.05
			MRS-L	0.124506±0.008	0.088933±0.005
			MRS-S	0.625±0.08	0.446429±0.05
			MRS-G	0.303995±0.02	0.21714±0.01
K47	cocci	37	MRS-F	0.267045±0.05	0.190747±0.04
			MRS-L	0.124506±0.008	0.088933±0.005
			MRS-S	0.590909±0.03	0.422078±0.02
			MRS-G	0.200926±0.03	0.143519±0.02
R57	cocci	37	MRS-F	0.278409±0.07	0.198864±0.05
			MRS-L	0.055336±0.02	0.039526±0.01
			MRS-S	0.357955±0.02	0.255682±0.01
			MRS-G	0.303995±0.02	0.21714±0.01
K14	cocci	30	MRS-F	2.221591±0.07	1.586851±0.05
			MRS-L	0.405138±0.01	0.289385±0.009
			MRS-S	1.528409±0.08	1.0917210.06
			MRS-G	0.712218±0.02	0.508727±0.01
K43	cocci	30	MRS-F	1.676136±0.04	1.19724±0.03

			MRS-L	0.464427±0.01	0.331733±0.009
			MRS-S	1.494318±0.02	1.06737±0.01
			MRS-G	0.958309±0.04	0.684507±0.03
K15	cocci	30	MRS-F	1.051136±0.04	0.750812±0.02
			MRS-L	0.373518±0.02	0.266798±0.01
			MRS-S	0.965909±0.08	0.689935±0.05
			MRS-G	0.552982±0.04	0.394987±0.03
R13	bacilli	30	MRS-F	0.363636±0.03	0.25974±0.02
			MRS-L	0.288538±0.01	0.206098±0.01
			MRS-S	1.034091±0.01	0.738636±0.01
			MRS-G	0.547192±0.03	0.390851±0.02
K17	cocci	30	MRS-F	0.619318±0.07	0.44237±0.05
			MRS-L	0.399209±0.005	0.28515±0.003
			MRS-S	5.340909±0.16	3.814935±0.11
			MRS-G	0.665895±0.04	0.475639±0.02
R6	cocci	30	MRS-F	1.073864±0.07	0.767045±0.05
			MRS-L	0.335968±0.02	0.239977±0.01
			MRS-S	1.198864±0.08	0.856331±0.06
			MRS-G	0.535611±0.02	0.382579±0.01
B3	cocci	30	MRS-F	1.403409±0.05	1.002435±0.04
			MRS-L	0.523715±0.01	0.374082±0.009
			MRS-S	0.840909±0.06	0.600649±0.04
			MRS-G	0.425594±0.02	0.303995±0.02
K40	bacilli	30	MRS-F	1.056818±0.04	0.75487±0.03
			MRS-L	0.448617±0.01	0.32044±0.01
			MRS-S	0.954545±0.06	0.681818±0.04
			MRS-G	0.683266±0.01	0.488047±0.011
B1	cocci	30	MRS-F	1.170455±0.04	0.836039±0.03
			MRS-L	0.318182±0.002	0.227273±0.001
			MRS-S	1.164773±0.04	0.831981±0.03
			MRS-G	0.836711±0.03	0.597651±0.02
B2	cocci	30	MRS-F	0.846591±0.07	0.604708±0.05
			MRS-L	0.288538±0.01	0.206098±0.01
			MRS-S	0.954545±0.06	0.681818±0.04
			MRS-G	0.66879±0.04	0.477707±0.03
K24	cocci	30	MRS-F	0.477273±0.03	0.340909±0.02
			MRS-L	0.049407±0.01	0.035291±0.009
			MRS-S	0.159091±0.06	0.113636±0.04
			MRS-G	0.376375±0.04	0.268839±0.02
T229	cocci	30	MRS-F	0.147727±0.04	0.105519±0.03
			MRS-L	0.079051	0.056465
			MRS-S	0.002	0.0015
			MRS-G	0.104227±0.01	0.074448±0.01
R11	cocci	30	MRS-F	0.488636±0.04	0.349026±0.03
			MRS-L	0.249012±0.01	0.177866±0.01

			MRS-S	1.051136±0.04	0.750812±0.02
			MRS-G	0.579039	0.413599
R10	cocci	30	MRS-F	0.704545±0.03	0.503247±0.02
			MRS-L	0.175889±0.02	0.125635±0.01
			MRS-S	1.113636±0.03	0.795455±0.02
			MRS-G	0.231616	0.16544
K13	cocci	30	MRS-F	1.068182±0.06	0.762987±0.04
			MRS-L	0.405138±0.01	0.289385±0.009
			MRS-S	0.926136±0.02	0.661526±0.017
			MRS-G	0.515345±0.008	0.368103±0.005
R7	cocci	30	MRS-F	1.872727±0.07	1.337662±0.05
			MRS-L	0.126482±0.01	0.090344±0.007
			MRS-S	1.261364±0.01	0.900974±0.01
			MRS-G	0.894615±0.03	0.639011±0.02
K27	bacilli	30	MRS-F	0.960227±0.07	0.685877±0.05
			MRS-L	0.316206	0.225861
			MRS-S	0.721591±0.05	0.515422±0.04
			MRS-G	0.37348±0.04	0.266771±0.03
R5	cocci	30	MRS-F	0.715909±0.04	0.511364±0.03
			MRS-L	0.365613±0.01	0.261152±0.01
			MRS-S	0.625±0.08	0.446429±0.06
			MRS-G	0.57324±0.008	0.409463±0.005
K41	bacilli	30	MRS-F	0.653409±0.04	0.466721±0.02
			MRS-L	0.055336±0.02	0.039526±0.01
			MRS-S	0.681818	0.487013
			MRS-G	0.541401±0.02	0.386715±0.02
R1	bacilli	37	MRS-F	1.107955±0.04	0.791396±0.02
			MRS-L	0.6917±0.02	0.494071±0.02
			MRS-S	0.954545±0.06	0.681818±0.04
			MRS-G	0.839606±0.04	0.599719±0.02
N1	cocci	37	MRS-F	0.863636±0.06	0.616883±0.04
			MRS-L	0.482213±0.01	0.344438±0.007
			MRS-S	0.852273±0.08	0.608766±0.057
			MRS-G	0.442965±0.05	0.316403±0.038
K49	bacilli	37	MRS-F	0.596591±0.04	0.426136±0.02
			MRS-L	0.217391±0.02	0.15528±0.02
			MRS-S	1.511364±0.04	1.079545±0.03
			MRS-G	0.405327	0.289519
K45	bacilli	37	MRS-F	0.846591±0.07	0.604708±0.05
			MRS-L	0.094862±0.02	0.067758±0.01
			MRS-S	0.44886±0.008	0.320617±0.005
			MRS-G	0.231616	0.16544
T78	cocci	30	MRS-F	1.659091±0.06	1.185065±0.045
			MRS-L	1.106719±0.05	0.790514±0.04
			MRS-S	0.857955±0.07	0.612825±0.05

			MRS-G	0.605096±0.04	0.432211±0.03
K51	cocci	37	MRS-F	1.210227±0.05	0.864448±0.04
			MRS-L	0.6917±0.02	0.494071±0.02
			MRS-S	1.539773±0.07	1.099838±0.05
			MRS-G	0.92067±0.008	0.657623±0.006
R81	cocci	37	MRS-F	1.971591±0.05	1.408279±0.04
			MRS-L	0.444664±0.01	0.317617±0.009
			MRS-S	1.068182±0.06	0.762987±0.045
			MRS-G	1.302837±0.04	0.930598±0.02
R96	bacilli	37	MRS-F	1.397727±0.04	0.998377±0.03
			MRS-L	0.523715±0.01	0.374082±0.009
			MRS-S	3.579545±0.08	2.556818±0.057
			MRS-G	1.876086±0.03	1.340061±0.02
R62	cocci	37	MRS-F	2±0.09	1.428571±0.06
			MRS-L	0.843874±0.01	0.602767±0.01
			MRS-S	1.988636±0.08	1.420455±0.05
			MRS-G	0.538506±0.02	0.384647±0.02
K34	cocci	30	MRS-F	0.943182±0.04	0.673701±0.03
			MRS-L	0.365613±0.01	0.261152±0.009
			MRS-S	0.329545±0.01	0.23539±0.011
			MRS-G	0.512449±0.01	0.366035±0.008
K42	cocci	30	MRS-F	1.159091±0.03	0.827922±0.02
			MRS-L	0.213439±0.02	0.152456±0.01
			MRS-S	0.744318±0.08	0.53165±0.066
			MRS-G	0.231616	0.16544
R8	cocci	30	MRS-F	0.721591±0.05	0.515422±0.04
			MRS-L	0.41502±0.02	0.296443±0.01
			MRS-S	0.255682±0.04	0.18263±0.02
			MRS-G	0.309786±0.02	0.221276±0.02
K39	bacilli	30	MRS-F	0.602273±0.04	0.430195±0.03
			MRS-L	0.322134±0.008	0.230096±0.005
			MRS-S	0.960227±0.07	0.685877±0.05
			MRS-G	0.425594±0.02	0.303995±0.02
K2	bacilli	30	MRS-F	0.409091±0.09	0.292208±0.06
			MRS-L	0.177866±0.02	0.127047±0.01
			MRS-S	0.568182	0.405844
			MRS-G	0.952519±0.03	0.680371±0.02
K10	bacilli	30	MRS-F	1.784091±0.04	1.274351±0.03
			MRS-L	0.517787±0.005	0.369848±0.004
			MRS-S	1.846591±0.04	1.318994±0.03
			MRS-G	0.431384±0.03	0.308131±0.02
K1	cocci	30	MRS-F	1.159091±0.03	0.827922±0.02
			MRS-L	1.245059±0.02	0.889328±0.01
			MRS-S	0.9375±0.12	0.669643±0.08
			MRS-G	0.086856±0.04	0.06204±0.02

R4	cocci	30	MRS-F	0.732955±0.07	0.523539±0.05
			MRS-L	0.494071±0.02	0.352908±0.01
			MRS-S	2.443182±0.08	1.74513±0.05
			MRS-G	0.914881±0.01	0.653487±0.01
K8	cocci	30	MRS-F	1.590909	1.136364
			MRS-L	0.604743±0.01	0.431959±0.011
			MRS-S	1.392045±0.04	0.994318±0.02
			MRS-G	0.773017±0.02	0.552155±0.02
R14	bacilli	37	MRS-F	2.102273±0.08	1.501623±0.05
			MRS-L	0.909091±0.05	0.649351±0.04
			MRS-S	1.3125±0.07	0.9375±0.05
			MRS-G	0.775912±0.04	0.554223±0.03
K7	cocci	30	MRS-F	1.619318±0.04	1.156656±0.02
			MRS-L	0.889328±0.02	0.635234±0.019
			MRS-S	1.232955±0.02	0.880682±0.01
			MRS-G	0.738274±0.02	0.527339±0.014
K9	cocci	30	MRS-F	1.193182±0.08	0.852273±0.057
			MRS-L	0.535573±0.02	0.382552±0.017
			MRS-S	1.982955±0.08	1.416396±0.06
			MRS-G	0.521135	0.372239

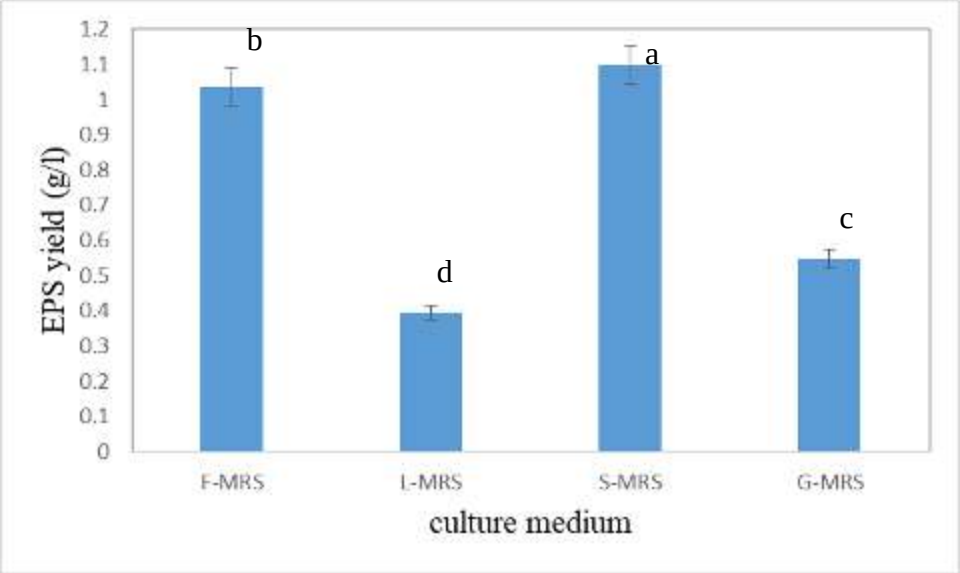


Figure 1- effect of carbon source on EPS yield

Analysis of data showed MRS broth medium formulated with sucrose had great effect on EPS production, but MRS broth formulated with lactose had a low impact on EPS synthesis. Kanmani et al (2013) showed that the production of EPS from *E. faecium* MC13 in the sucrose medium was higher than in lactose, glucose and fructose media which was 11.33 g/l (Kanmani et al., 2013). In other work Kanmani et al (2011) expressed the maximum yield of EPS from *Streptococcus phocae* PI80 (11.75 and 12.14 g/L) was obtained in the presence of lactose and yeast extract at a concentration of 20 g/l (Kanmani et al., 2011). Knowledge of the effect of the sugar source on EPS production and the activities of biosynthetic enzymes

provides information about the mechanisms of regulation of the synthesis of EPS which can contribute to improve polymer production (Mozzi et al., 2001).

Conclusion

Food industry is looking for the multifunctional strains of LAB that contribute to the organoleptic, technological, nutritional and health properties of fermented dairy products. EPS producing lactic cultures have tremendous potential as functional starters, which can be better substituted to many commercial additives in use. However, EPS producing character is plasmid associated in LAB and may be lost over generations. Further it varies from strain to strain. Hence, selection of promising strains that retain EPS producing characteristic over a long period, may give opportunities to food manufacturer to produce various low-fat products. Fermented dairy products, especially made by traditional method in rural areas, are the potential reservoir for isolation of EPS producing cultures.

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LEGUME COVER CROPS EFFECTIVENESS FOR WEED CONTROL IN THE MEDITERRANEAN REGION

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Abstract

The European Commission presented recently its' new strategy for the defence and preservation of biodiversity. This contemplates the adoption of a "Farm to Fork" strategy, aiming to create a sustainable production and consumption system. The EU presents measures leading to a 50% reduction in the use of plant protection products by 2030, which makes necessary the development of alternative practices such as adopting non-chemical weed management. The use of cover crop is expected to control weed infestation and reduce the need of herbicide application. The choice of cover crop species adapted to the local conditions and providing a large amount of biomass is critical as weed control capacity is linked to cover crop soil cover density. In this study held in central Portugal, 6 species of legumes (Forage Pea, Yellow Lupin, Red Clover, Balansa Clover, Persian Clover, Arrowleaf Clover) cultivated in the fall to spring period in succession with grain maize were tested and weed control performance was related with total dry matter production and soil fertility. Weed control efficiency was in fact clearly related with legumes biomass, clover species showing the best results in term of dry biomass in particular Arrowleaf and Balansa Clover with production above 8 t/ha and weed infection rating from 0 to 8% for optimum soil fertility conditions. Red clover showed also good performance although a weaker biomass production was compensated by precocity of developing. Soil fertility level was identified as an important indicator, a decrease in soil fertility led to a delay and a lower production of legume biomass compromising the effectiveness of legumes for weed control.

Keywords: *Cover crop, legumes, weed control, soil fertility.*

Introduction

The European Commission presented recently its' new strategy for the defence and preservation of biodiversity. A proposal that contemplates the adoption of the "Farm to Fork" strategy, aiming to create sustainable production and consumption systems, in addition to promote and safeguarde the planet's biodiversity. The EU points towards a 50% reduction in the use of plant protection products by 2030, which makes necessary to optimize tools, developing alternatives that protect and promote biodiversity such as adopting non-chemical weed management. Following the 2015 IARC report on glyphosate, many countries already decided to restrict or ban the use of this pesticide, and many others are considering restrictions in the use of active substance for the future.

The use of cover crop is one of such alternative practice that is expected to control the weed infestation and reduce the need of herbicide application. (Alonso-Ayuso et al., 2018; Brust et al., 2014; Buchi et al., 2020; Dorn et al., 2015). In addition, cover crops increase agroecosystem services by controlling soil erosion (De Baets et al., 2011), improving water retention (Hubbard et al., 2013), improving soil organic matter content (Raphael et al., 2016), reducing nutrient leaching (Gabriel et al., 2012), having a positive effect on nutrient balance

(Thorup-Kristensen et al.,2012; Plaza-Bonilla et al., 2016) and a positive impact on the microbial community (Brennan et al., 2017). The choice of cover crop species adapted to the local conditions and providing a large amount of biomass is critical as weed control capacity is usually linked to cover crop soil cover density (Reberg-Horton et al., 2012) and the precocity of it establishment (Melander et al., 2013; Dorn et al., 2015).
It is indispensable to test locally and assess the effectiveness of cover crop species to provide tools to advisories entities on how to manage efficacy a weed control strategy.
The main objective of the study is to test the effectiveness in term weed control of 6 species of legume and identify the most suitable species. The specific objectives were to identify the key parameters that determine the success or failure of cover crops in term of weed control, relationship between cover crop species, cover crop biomass, weeds biomass, weeds percentage and soil fertility.

Material and Methods

Study Site
The study area is located in the lower Mondego valley, an alluvium plane located in Central Portugal traditionally used for irrigated agriculture (maize and rice). Soils are modern alluvial soils, with a texture from silt-loam to sandy-clay-loam. Climate is Mediterranean, characterized by rainy winters and dry summers. The annual average temperature is 16.1ºC, with smooth variations. The annual average precipitation is 922 mm, occurring essentially between October and March.
Experimental design
Under the scope the international H2020 SoilCare project, the study was conducted in 2019-2020 at the Baixo Mondego experimental Center in Central Portugal, to evaluate the productivity and the weed control capacity of 6 species of legumes cultivated as winter cover crop and use as green manure for grain maize production in May.
The fall to spring period was exceptionally wet for November and December months with total monthly amount around 250 mm leading to sporadic waterlogging of the soils and for a rainfall event with more than 110mm in 19-20 of December that completely immersed the trial field during 36h. (figure 1a)

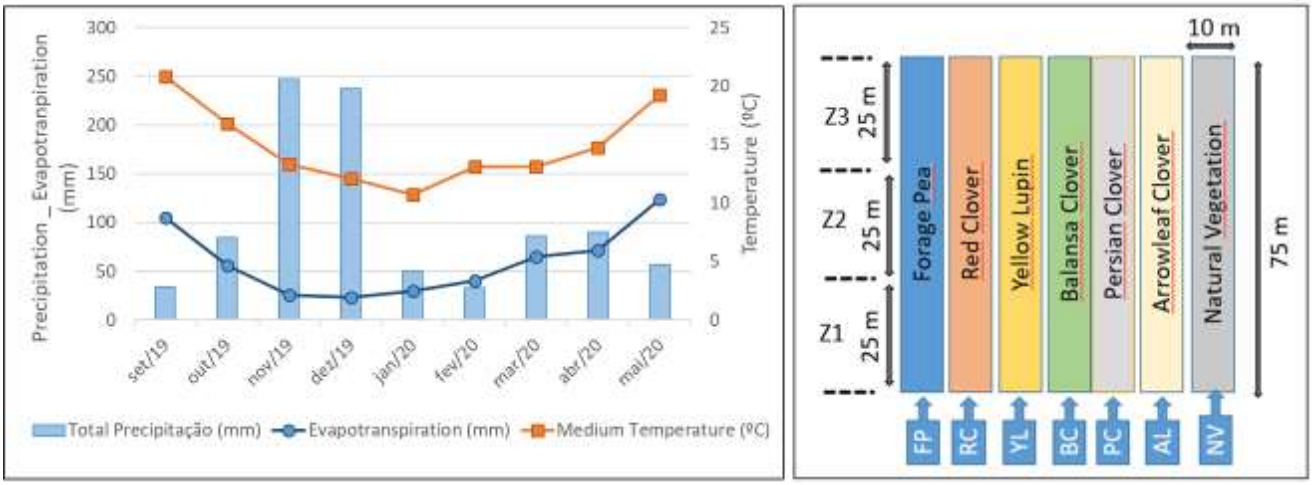


Figure 1a. Monthly precipitation, Average temperature and Evapotranspiration during the study period ; **1b.** Experimental design of the field trial.

The experimental design was composed of 7 plots that included 6 treatments plots: Forage Pea_FP (*Pisum sativum L*); Yellow Lupin_YL (*Lupinus Luteus*), Red Clover_RC (*Trifolium*

pratense); Balansa Clover_BC (*Trifolium Michelianum*); Persian Clover_PC (*Trifolium suaveolens*) Arrowleaf Clover_AC (*Trifolium vesiculosum*) and one control plot: Natural Vegetation_NV. Each plot had an area of 750m2 (10m*75m) and was divided in 3 sub-plots of 25*25m (Z1. Z2 and Z3) in order to take in account the large spatial variability of the soil fertility in the discussion of the results (figure 1b).

Crop management

Legumes were sown as winter cover crop in 03/12/2019 following the principal crop, a grain maize FAO 300 harvested in 04/10/2019. Legume cover crops were cut at full /flowering stage corresponding to different dates in function of the species' precocity. After cutting, crop residues were let at the soil surface and incorporated into the soil at the same date, 07/05/2020, before sowing the main culture, a grain maize FAO 300 in 20/05/2020. Soil preparation before cover crop seeding was 2 passes of disk harrow, to destroy and burrow into the soil maize residues and 1 pass of rotary hoe to prepare the seeding bed. Legumes seeds were broadcast seeding by hand. Seeding density are indicated in the table 1. Any fertilization or pesticides were applied before or during the cover crop growing period. (table 1).

Table 1. Agronomics parameters for the 6 cover crop species.

	Latin name	Code	Comercial name	Seeding rate (kg/ha)	Sowing date	Cutting date	Growing days
Forage Pea	*Pisum sativum*	FP	KAYNNE	60	03/12/2019	02/04/2020	121
Red Clover	*Trifolium pratense*	RC	CONTEA	30	03/12/2019	23/04/2020	142
Yellow Lupin	*Lupinus luteus*	YL	MISTER	60	03/12/2019	02/04/2020	121
Balansa Clover	*Trifolium michelianum*	BC	PARADANA	30	03/12/2019	23/04/2020	142
Persian Clover	*Trifolium suaveolens*	PC	LIGHTNING	25	03/12/2019	07/05/2020	156
Arrowleaf Clover	*Trifolium vesiculosum*	AC	ZULU II	35	03/12/2019	07/05/2020	156
Natural vegetation	* * *	NV	* * *	* * *	* * *	02/04/2020	121

Data collection

Vegetation samples were performed just before cutting the legumes cover crops. It was used the quadrats sampling methods. The area of the quadrat was 0.5m2 and for each plot 12 samples of vegetation (corresponding to 6m2) were collected (4 in each of the 3 zones: Z1 Z2 and Z3). Samples were then weighted to determine the green biomass weight. For each sample, legumes and weed were sorted out and weighted separately. Both legumes and weed samples were dried during 72h at 65ºC, to determinate the dry matter content and total dry biomass.

Soil samples was collected on the 22nd April 2020 at a soil depth of 0-30cm for each sub plot in order to determine overall soil fertility. Organic matter content, N total, available P and K were analyzed at the laboratory.

Data analyses

Pearson's correlation coefficient was used to measure the statistical relationship between cover crop species, cover crop biomass, weeds biomass, weeds percentage and soil fertility.

Soil fertility coefficient was determined attributing to each of the 4 soil parameter considered in this study (MO, N total, Available P2O5 and K2O) a valor between 1 and 3 (1:low; 2:medium; 3:high fertility) and then adding the 4 values a soil fertility coefficient comprised between 4 and 12 was obtained.

Results and Discussion

Cover crop biomass production

Overall cover crops biomass production varies widely with the species and the local characteristics of where they are planted (Z1, Z2 or Z3, ie soil fertility). Due to significant variation in dry matter content between species, the relationship between green biomass and dry biomass is not constant as presented in the table 2.

Considering the median biomass production, clovers species present the best yields specially arrowleaf and balansa clover respectively with 61.5 and 47.5 t/ha of green biomass or 8.9 and 7.5 t/ha of dry biomass. The forage pea and yellow lupin present the weaker production of biomass respectively 9.0 and 29.5 t/ha of dry green biomass or 2.0 and 3.3 t/ha of dry biomass.

The extremely low biomass production of forage pea is explained by it high sensibility to water ponding, that happened during the installation phase of the cover crop and caused the death of seeds, decreasing drastically the production yield.

The yellow lupin entered in full flowering phase precociously after 121 days of growing and then was cut earlier that the clovers. This may explain the reduced biomass production of yellow lupin when compared with the other species.

All the clover species presented high median green biomass production in excess of 40 t/ha, with a maximum production above 60 t/ha for arrowleaf corresponding to almost 9 t of dry biomass for 156 growing days. Those are values extremely high values, when compared with the expected results.

Balansa clover presented also an exceptional behaviour and although a precocious flowering and a cut after 142 days, it produced 47.5 t/ha of green biomass or 7.5 t/ha of dry biomass.

These results demonstrated that excepted for forage pea, all the species of legumes tested were suitable to produce high level of dry matter, which is in line with the results of Alonso-Ayuso et al., 2018; Brust et al., 2014; Buchi et al., 2020, that rarely overpass 6 t/ha.

Weed control capacity.

In term of weed control performance, two species, arrowleaf and red clover presented high efficiency in the control of the large variability of weed presented in the field. (Hop Clove (*Medicago lupulina*), crown Daisy (*Chrysanthemum coronarium*) roman chamomile (*Chamaemelum mixtum*), corn spurry (*Spergula arvenses*) wild radish (*Raphanus raphanistrum),* sorrel (*Rumex induratus*) opium poppy (*Papaver somniferum*). They maintained weed production under 2t/ha instead of almost 15 for the control and infection yields inferior to 3% of the total biomass production. Balansa clover also presents satisfying results with 3.5 ton/ha of weeds representing 7% of total biomass. Nevertheless, the reasons of the efficiency differ between species. Red clover was the specie with the more regular emergence of plants and didn't suffer too much from the soil ponding allowing a rapid initial installation of the clover. It occupied early the soil surface and limited the emergency of weeds although presenting a limited production of biomass. In the case of arrowleaf and balansa clover the installation of the plants was slower and allowed some weed emergence in a first phase. However, the extremely high biomass production leaded to decumbent stems that formed a dense and thick soil cover that prevented almost totally the emergence of weeds.

Yellow lupin and Persian clover were not particularly effective in controlling weeds, they presented weed biomass similar to the control respectively 14.7 and 11.8 t/ha.

Forage pea was also inefficient in controlling weeds principally due to the high lethality of the seeds.

Although lasting only one year, this study allows to understand the level of efficiency of each species and the mechanism that are hidden behind them and confirmed the conclusions of Melander et al., 2013 and Dorn et al., 2015 that evocated the overwhelming importance of successful early stage installation of the cover crop at the soil surface and of Reberg-Horton et al., 2012 that highlighted the importance of a high level of productivity allowing an efficient competition for resources.

Relation between biomass production and weed control efficiency.

Considering the overall biomass production of legumes and weeds, there is a week negative Pearson correlation between legume and weed biomass (-0.63) but a stronger correlation between legume biomass and weed percentage (-0.91) pointing to the importance of the

species. This discrepancy disappears when the Pearson correlation is applying by specie. arrowleaf and balansa clover presented the higher negative correlation between legume and weed biomass production (>-0.95). In the case of red and persian clover, yellow lupin the correlation is weaker, the weed control capacity is not so clearly related to the legume production. In the case of forage pea, the correlation is positive, the biomass of weed increase with the biomass of pea.

This results imply that for species with rapid initial installation, the production of biomass is not as important than for species with later emergency. The control of weed is made at an early stage, and stay under control during the entire growing phase, which is not the case for clovers. These depend on the production of a very high amount of biomass to compete and control in a later phase the emergency of weeds.

Relation between biomass production and soil fertility.

Soil fertility play an important role in legume biomass production. In fact soil with low fertility coefficient (<5) presented severe decay in biomass production, leading to a decrease in weed control. It's the case of the 2 most productive species arrowleaf and balansa clovers that loss more than 50% of the legume biomass production under poorest soil conditions. Persian clover also suffered a decrease in biomass production but not so accentuate. The others species didn't present clear relation between soil fertility and legume biomass production, but in this experiment there were only under medium and good fertility conditions.

The result demonstrate that some species are more adapted to poor soil conditions as others failed completely in term of biomass productivity. This is extremely relevant in what concerns the choice of specie by the farmers, in function of the fields'soil fertility level. Nevertheless, legumes incorporation increasing soil fertility at the long term, the choice of the species can be re-evaluated through time.

Table 2. Green and Dry Biomass production of Legumes, Weeds and Total (legumes + weeds) in spring before cutting for the 3 zones (Z1, Z2 and Z3) and soil fertility parameters.

		Green Biomass				Dry Biomass				Organic	Total	Available		Fertility
		TOTAL	Legumes	Weed	Weed	TOTAL	Legumes	Weed	Weed	Matter	N	P_2O_5	K_2O	Coeff
		ton/há	ton/há	ton/há	%	ton/há	ton/há	ton/há	%	%	mg/kg	mg/kg	mg/kg	
Forage Pea	Z1	40,7	14,8	25,9	64%	6,9	3,3	3,6	52%	2,1	1312	374	171	11
	Z2	17,6	9,0	8,6	49%	3,2	2,0	1,2	37%	1,9	1102	339	181	11
	Z3	13,6	6,5	7,1	52%	2,4	1,5	1,0	40%	1,8	1012	131	133	6
	Average	*24,0*	*10,1*	*13,9*	*55%*	*4,2*	*2,3*	*1,9*	*43%*					
	Media	**17,6**	**9,0**	**8,6**	**52%**	**3,2**	**2,0**	**1,2**	**40%**					
Red Clover	Z1	64,3	42,5	5,8	9%	7,1	6,3	0,8	12%	2,0	1182	347	156	11
	Z2	66,3	48,0	1,8	3%	7,4	7,1	0,3	3%	1,9	1111	307	188	11
	Z3	60,7	43,5	2,0	3%	6,7	6,4	0,3	4%	1,7	1098	135	165	8
	Average	*63,8*	*44,7*	*3,2*	*5%*	*7,1*	*6,6*	*0,5*	*7%*					
	Media	**64,3**	**43,5**	**2,0**	**3%**	**7,1**	**6,4**	**0,3**	**4%**					
Yellow Lupin	Z1	46,4	34,7	11,7	25%	5,3	3,9	1,4	27%	1,9	1187	267	279	12
	Z2	35,8	20,9	14,9	42%	4,1	2,3	1,8	43%	1,9	1152	272	247	12
	Z3	44,2	29,5	14,7	33%	5,0	3,3	1,8	35%	1,6	987	128	214	8
	Average	*42,1*	*28,4*	*13,8*	*33%*	*4,8*	*3,2*	*1,6*	*35%*					
	Media	**44,2**	**29,5**	**14,7**	**33%**	**5,0**	**3,3**	**1,8**	**35%**					
Balansa Clover	Z1	51,0	47,5	3,5	7%	8,1	7,5	0,6	7%	1,9	1195	283	179	11
	Z2	65,0	62,0	3,0	5%	10,3	9,8	0,5	5%	1,9	1220	179	160	10
	Z3	32,3	24,8	7,5	23%	5,2	3,9	1,3	24%	1,5	927	132	106	5
	Average	*49,4*	*44,8*	*4,7*	*12%*	*7,9*	*7,1*	*0,8*	*12%*					
	Media	**51,0**	**47,5**	**3,5**	**7%**	**8,1**	**7,5**	**0,6**	**7%**					
Persian Clover	Z1	46,2	42,8	3,4	7%	7,2	6,4	0,7	10%	1,9	1226	224	159	11
	Z2	54,5	42,7	11,8	22%	9,0	6,4	2,6	29%	2,0	1248	180	172	10
	Z3	43,1	29,8	13,3	31%	7,4	4,5	2,9	40%	1,4	875	149	115	4
	Average	*47,9*	*38,5*	*9,5*	*20%*	*7,9*	*5,8*	*2,1*	*26%*					
	Media	**46,2**	**42,7**	**11,8**	**22%**	**7,4**	**6,4**	**2,6**	**29%**					
Arrowleaf Clover	Z1	61,5	61,5	0,0	0%	8,9	8,9	0,0	0%	1,9	1216	194	188	10
	Z2	66,2	64,0	2,2	3%	9,8	9,3	0,5	5%	1,9	1209	125	179	9
	Z3	29,6	21,2	8,4	28%	4,9	3,1	1,9	38%	1,3	837	152	105	4
	Average	*52,4*	*48,9*	*3,5*	*11%*	*7,9*	*7,1*	*0,8*	*14%*					
	Media	**61,5**	**61,5**	**2,2**	**3%**	**8,9**	**8,9**	**0,5**	**5%**					
Natural vegetation	Z1	18,7	0,0	18,7	100%	3,3	0,0	3,3	100%	2,0	1276	193	192	10
	Z2	14,7	0,0	14,7	100%	2,6	0,0	2,6	100%	2,0	1218	145	173	9
	Z3	6,4	0,0	6,4	100%	1,1	0,0	1,1	100%	1,5	938	167	133	6
	Average	*13,3*	*0,0*	*13,3*	*100%*	*2,4*	*0,0*	*2,4*	*100%*					
	Media	**14,7**	**0,0**	**14,7**	**100%**	**2,6**	**0,0**	**2,6**	**100%**					

Table 3. Pearson's correlation coefficient between Legumes Biomass (LB) Weeds Biomass (WB), Weed Percentage (WP) and Soil Fertility (SF)

	LB/WB	LB/WP	LB/F	WB/F	WP/F
Forage Pea	0,98	0,88	0,73	0,56	0,31
Red Clover	-0,68	-0,71	0,34	0,45	0,42
Yellow Lupin	-0,81	-0,99	-0,14	-0,47	0,00
Balansa Clover	-0,96	-0,96	0,85	-0,97	-0,96
Persian Clover	-0,62	-0,80	0,99	-0,71	-0,87
Arrowleaf Clover	-0,95	-0,99	0,98	-1,00	-1,00
TOTAL	-0,63	-0,91	0,28	0,05	-0,16

Conclusions

This study addresses the potential of 3 species of legumes (arrowleaf, balansa and red clover) for weed control, reducing the infestation of weed at less than 5 % of the total biomass for optimal soil conditions. Arrow leaf and balansa clover efficacy in weed control is due to a huge production of green biomass up to 60 ton/ha or the equivalent to 9 ton/ha of dry matter with decumbent stems, able to stop weeds development. Red clover despite a lower biomass production is also efficient in weed control due to its early installation occupying the soil very quickly and strongly. Nevertheless, it seems that the more productive species suffered more in case of adverse conditions of soil quality and could not be adapted to weaker soils. Red clover presented a better resilience to low soil quality conditions and could be more adapted to poor fertility conditions. Besides the weed control effectiveness during the winter, the high biomass production will allow, in the case of no tillage system, the efficient use of cover

crops residues at the soil surface as mulch limiting weeds emergence and providing an important source of nutrients for the cash crop.

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MECHANICAL QUANTIFICATION OF HEAVY METAL CONTAMINANTS IN HAMMER-MILLED FLOUR AND MILL DESIGN FOR ENHANCED FOOD SAFETY

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Abstract

In an attempt to enhance food safety, heavy-metal contaminants were mechanically quantified in hammer-milled flour compared to hand pounding (Hp). Hp samples, control treatment, was produced by pestle and mortar and compared to LB7-machine type, main treatments-foundry hammer mill (FHm) and artisanal hammer mill (ArtHm). The machine types were also compared. All machine types were experimented in Malawi whereas Mozambique tested FHm only. ZM523 Maize, (*Zea mais)* Hp flour sample was used whereas main treatment flour samples were from experimental hammer mills. Then, samples were analyzed by Atomic Absorption Spectrophotometry (AAS) in *ppm* and compared to recommended limits. Eight metal elements as Aluminum (Al), Zinc (Zn), Arsenic (As), Cadmium (Cd), Copper (Cu), Lead (Pb), Manganese (Mn) and Iron (Fe) were measured. Weight loss of beaters of machine types was also determined and then samples magnetically metal-scanned. The results, machine types achieved higher metal contents than Hp with whole maize grain flour sample ranging from 28.60±0.80 to 85.24±1.21, 35.60±0.60 to 562.05±4.11 and 0.07±0.1 to 19.18±4.0 *ppm* for FHm, ArtHm and Hp, respectively. Mozambique achieved similar results comparing 38.50±0.60 to 802.0±1.44 *ppm* to Hp. For machine type, in Malawi, ArtHm achieved much higher concentration than FHm. Much higher wearing rates of beaters of 75.02 ppm for ArtHm than 60.48 *ppm* FHm were recorded. For metal-contamination, all machine types achieved higher than the control. In conclusion, more metal-contaminants than recommended limits by the current hammer-mill designs, is confirmed and thus urgent need to reduce them by hammer mill redesign to enhance food safety.

Keywords: *hammer-milled flour, metal contaminants, enhanced food safety, hand pounding, health hazard.*

Introduction

Malawi and Mozambique predominantly grow maize (*Zea mais*) crop as staple food. After harvest, maize grain is processed into flour. Food processing involves grinding of food material called size reduction into flour. Grinding (Ehiri *et al*,2010) in the past was done using pestle and mortar methods, (Yahaya *et al.*, 2012) but these days, modern methods such as hammer mills have been developed which when in operation the hammer milling beats the grain into powder where the milling process leads to wear and tear of the beaters thereby introducing contaminants into the flour (Cheftel, 1988, Sule and Odugbose, 2014). The presence of heavy metals in food is highly significant for they are capable of causing serious health problems depending on the nature of the heavy metals. Some of these metals such as lead (Pb), mercury (Hg) and arsenic (As) are linked to human poisoning (Passwater and Cranton 1993, Gerstner and Huff, 1997, Dibofori-orji and Edori, 2013) by accumulation in the brain, kidneys and immune system and severely disrupting normal body functions.

Despite of these lamentations, the use of hammer milling to produce flour is all over in Southern Africa Developing Community (SADC) such as Malawi and Mozambique and has become an economically attractive activity in both urban and rural areas (Dallatu *et al.*, 2016). Figure **(Fig.) 1** in sections **1** to **4,** depicts the process of how hammer mill beaters undergo tear and wear during milling operation. In this case and when brand new, the beaters are rectangular-shaped (as shown in **section 1)** but their condition, after milling for some time shows excessive metal loss as in **section 4** (MIT, 1999).This is due to knocking-off of metal chips by heavy agitating activity of the milling process and these chips are blown together with the flour and collected together with the flour at the cyclone. In the present study, food safety was described as handling and preparation of food in such hygienic ways where metal contents are comparable to recommended set limits by World Food Program (2011) and FAO /WHO, (1983) (Table **1)** so as to prevent food borne illness from diarrhea to various forms of cancer (Mridha, 2013).

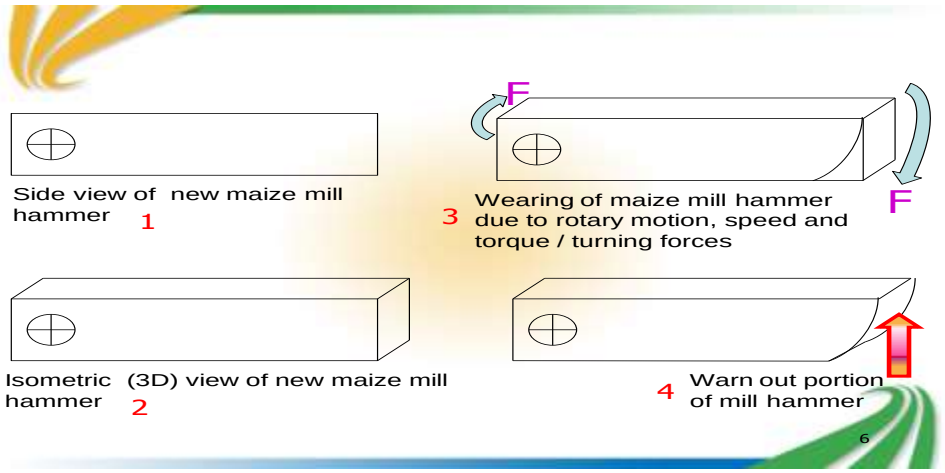


Fig. 1. Process of Metal loss due to hammer milling.

Table 1: Specific maximum limits of heavy metal contaminants in Maize flour and Fish

Heavy metal Contaminants	**Maximum limits of metal contaminants [ppm]**	
	Maize Flour (WFP, 2011)	Fish (FAO /WHO, 1983)
Arsenic (As)	0.10	-
Copper (Cu)	2.00	-
Lead (Pb)	0.10	-
Cadmium (Cd)	0.02	-
Mercury (Hg)	0.01	-
Zinc (Zn)	-	40.0
Tin (Sn)	-	50.0

Source: World Food Program -WFP, (2011), and FAO /WHO, (1983)

For the present study, heavy metal contamination in hammer-milled flour were considered as basic metals such as lead (Pb), cadmium (Cd), copper (Cu), iron (Fe), arsenic (As), manganese (Mn), zinc (Zn), and Aluminum (Al) toa major global concern in the ecosystem (Somers, 1974, Cheftel, 1988 and Normanyo *et al* 2010). Furthermore, studies by Järup, (2003) added that toxic heavy metal of Cd exposure causes bone effects and fractures, Pb makes humans particularly susceptible to high gastro-intestinal uptake, permeable blood-brain barrier and causes neurotoxic effects, and just as AS leads to increased risks of skin cancer and lesions such as hyperkeratosis and pigmentation changes and may increase chances of

lung cancer. As such our study recognized that a lot of studies on food processing by the milling equipment (Dallatu *et al.*, 2016, Jolly *et al.,* 2013), however, until presently no successful and elaborate mechanical quantification of heavy metal contaminants strategic to minimize flour contamination and optimizing food safety by redesigning the current hammer mill design to reduce the metal contamination (Ofori *et al.*, 2016; Järup, 2003), has been reported and thus heavy-contaminants in the food chain in the SADCC region. In this case the purpose of our study was to elaborately quantify the heavy metals in the hammer-milled and recommend hammer redesign that would reduce the metal contaminants, in flour, for enhanced for food safety.

Materials and Methods

Various procedures were followed. The experimental materials such as hammer mills as machine type for main treatments and traditional pestle and mortar, as control were used. Experimental LB7 machine type were defined as Artisanal hammer mill (ArtHm) in **Fig. 2a** which is locally made by the artisans and its fabrication uses old scrap metal pieces, cut and welded together (**Fig.2b).** and as foundry hammer mill- FHm (**Fig.3**)**.** LB 7 has 26 beaters and is the popular size and choice by most investors. The both machine types are equipped with mild steel beaters for milling. However, ArtHm due to scrap metal pieces, the ArtHm, is hypothesized to have higher metal-contaminants than FHm type depicted in **Fig. 3**. In our study all machine types were hypothesized as grinding equipment (ArtHm and FHm) to have more flour contamination than hand pounding (Hp) due to the mentioned metal chippings leading to metal addition.



Fig.2a Typical LB-7 ArtHm



Fig.2b Fabrication of typical ArtHm.



Fig.3 Typical LB-7 FHm

FHm design is sim In terms of methods, identification of hammer mill types and study sites, were carried out in selected areas in each country. In Malawi, two pilot districts (Lilongwe and Salima) were identified where two machine types were experimented with one at Chitedze (FHm) and the other at Msundwe Trading Centre (ArtHm) in Lilongwe. In Salima, the same was done at Salima Town Centre and Tembwe Extension Plan Area (EPA), respectively. A structured questionnaire was used for site and machine type identification. In Mozambique, the activity was conducted at Nampula City where FHm only as machine type, was experimented. In each case, a randomized complete block design (RCBD) with three treatments was used. The treatment combinations were defined as (a) maize flour samples produced by Hp as control treatment and (b) flour samples produced by hammer milling (Hm)-machine type as main treatment. In all this grain flour produced by ArtHm and FHm were further defined as machine type sub-treatments. Hp control treatment was technically conducted in Malawi, only. For the control treatment, eight Hp grain samples (Table 2a) were prepared (Fig.4) and OPV 523 maize variety, supplied by Chitedze Maize Commodity Team, was used. For Hp, all samples were processed as defined in Table 2 column (a) where non-metallic containers or utensils were used and two causal workers were engaged. Then after Hp pounding, each sample was sieved by winnowing and tossing using bamboo basket called

Lisero, until fine quality flour was obtained. For dehulled-soaked maize or white flour, Ufa woyera, the samples were soaked for three days in plastic bucket and then processed by Hp into white flour. In all, eight Hp flour samples were prepared. Then the flour samples (FHm and Art Hm) were processed as received by customers and as defined in Table 2 column (b) where nine samples were prepared and collected from each of the hammer mill type



Fig.4.Sample preparation by hand pounding using pestle and mortar method



Fig. 5 Weighing of grain sample prior to milling.

At each site, the procedure for data collection was that each sample was first weighed (**Fig. 5**) prior to milling to determine the sample initial quantity prior to milling. Then, the sample was put into the hammer mill whereupon the milling process, three random flour samples were drawn at defined intervals as at the start of the milling, mid-way and at the end with total recalibrated weight averaging 200 g per scooped sample lot. This procedure was applied to the rest of the subsequent samples and similarly handled and separately kept in sample bags, according to the sample type, machine type and composition in **Table 2** column **(b)**. However, for this treatment an additional sample designated as, *Emergency* sample no.**9**, was added. This one was realized when the grain sample was accidently mixed with metal piece during milling operation. As such sample no.**9** assumed to contain more metal contaminants than the other ones. As for Mozambique the procedure for flour sample collection was similar to Malawi.

In terms of weight loss of beaters, an assessment was conducted based on the beaters' weight reduction as milling progressed over time. In the weight loss determination at each hammer mill, the beaters were firstly weighed prior to installation and hammer mill operation, then weight loss of beaters checked at regular intervals, weighed and turned around for every 11 to 14 days until all beater corners (**Fig.1)** were clearly worn out. Thereafter, the first set of worn out 26 beaters was replaced with brand-new ones. This was replicated over ten times. Furthermore, flour samples were drawn from each machine type daily when the hammer mill was in operation. Then the metal contents in the flour samples were quantified by Atomic Absorption Spectrophotometry (AAS) analysis expressed in parts per million (ppm). Such heavy metals as Al, Zn, As, Cd, Cu, Pb, Mn and Fe were analyzed where the data set was averaged and plotted on a graph.

Table 2: (a) Sample preparation by hand pounding with traditional pestle and mortar and (b) Samples preparation by hammer milling (both ArtHm and FHm)

No	(a) Flour sample type by Hp	(b) Flour sample type by ArtHm and FHm
1	Whole grain maize flour "*Mgaiwa*"	Whole maize grain, *Mgaiwa*, flour
2	Dehulled Soaked maize / white flour "*Ufa woyera*"	Dehulled and unsoaked maize / Grain meal flour
3	Dehulled unsoaked maize / Grain meal flour	Dehulled Soaked maize / white *Ufa woyela*, flour

4	Maize bran, *Gaga* or *madeya*, flour	Maize bran, *Gaga* or *madeya*, flour
5	Soaked and fermented maize, *Chimera*, flour	Mixture of soybean and maize flour
6	Mixture of soybean and maize flour	Mixture of soybean, maize, sorghum rice and groundnut flour
7	Mixture of soybean, maize, sorghum, rice and groundnut flour	Mixture of soybean, maize, sorghum and groundnut flour
8	Mixture of soybean, maize, sorghum and groundnut flour	Soaked and fermented maize, *Chimera*, flour
9	-	Emergency sample of whole maize grain, *Mgaiwa*, flour

As strategy for metal removal the flour samples were metal detected by scanning using hand-magnet at Chitedze Agricultural Research Station Lab in Malawi. Prior to magnetic scanning each sample was put in centrifuge bottle for shaking using machine shaker for 10 minutes (min) at 220 oscillations per min. and then put to rest for 2 min. Thereafter, the samples were centrifuged for 5 min at 5000 rpm, then stopped and left to settle for 2 to 3 days. Then the flour at the bottom portion of the contents extracted and spread on white sheet of paper and subjected to surface hand-magnetic scanning and the trapped metal chippings were visually observed, using magnifying glass, and recorded in **Table 5**, below.

Results and Discussions

The results in **Table 3** depict the findings for heavy metal contaminants for hammer milling (FHm), in Mozambique compared to Hp in Malawi. In this case it was shown that Hp achieved lower metal contents confirming no metal addition by hand pounding /processing. However, FHm performance for **Table 3**, compared to the same for Malawi (**Table 4**), showed much higher concentration, respectively comparing As, Cu, Pb and Cd: 57.80±0.64, 62.83±0.50, 471.43±9.01 and 802.0±1.44 ppm for Mozambique in Serial no.1 against **Table 4** for metal contents of 54.85±0.60, 54.85±0.60, 421.47±9.01, 85.24±1.21 ppm in Malawi. In all this Hp scored lowest metal contents showing no metal contamination.

Table 3: Comparison of Metal contents FHm Flour in Mozambique with Hp Flour in Malawi.

sample	**Metal content [ppm] wet weight mean ± SEM, FHm vs Hp method**															
	FHm **(Mozambique)**								**Hp (Malawi)**							
	Zn	**Cu**	**Mn**	**Fe**	**Al**	**As**	**Pb**	**Cd**	**Zn**	**Cu**	**Mn**	**Fe**	**Al**	**As**	**Pb**	**Cd**
1	38.50±0.60	62.83±0.50	502.95±6.11	372.25±6.01	39.90±0.95	57.80±0.64	471.43±9.01	802.0±1.44	19.18±4.0	0.26±0.02	1.07±0.90	8.23±0.70	4.18±4.0	0.36±0.12	3.08±0.12	0.07±0.1
2	28.10±2.01	48.66±8.13	295.01±7.12	169.31±3.07	26.12±2.11	38.05±5.12	306.07±6.02	102.32±1.91	13.88±3.0	0.47±0.82	2.7±0.42	7.43±0.21	3.91±3.00	0.57±0.98	0.90±4.12	0.00
3	18.23±1.77	33.54±0.16	1.09±7.31	99.04±2.61	22.56±4.62	26.23±3.01	30.10±9.11	72.61±0.13	9.14±1.1	1.48±0.03	1.84±0.10	43.3±5.9	2.09±2.10	0.43±9.10	0.03±1.02	0.34±0.15

1 denotes Whole maize grain, *Mgaiwa*, flour, 2 denotes Dehulled and unsoaked maize / Grain meal flour, 3 denotes Dehulled Soaked maize / white flour or *Ufa wovela*.

As discussed in the preceding section, higher metal contents by FHm in Mozambique, was

In the same respect **Table 4**, depicts the results for assessment of metal content in Hm samples compared to Hp flour samples, in Malawi. In these results (**Table 4**) show variation of metal contents (wet weight mean ± SEM) in flour samples with method of flour production. The trend of results revealed that for the milling equipment used in this study, ArtHm introduced higher metal contaminants into food than FHm. For example, in sample with **Serial no. 1** (Table **4**) flour, it was observed that much higher levels from ArtHm for As, Cu, Pb and Cd averaged 72.55±0.44, 74.85±0.34, 589.01±6.21 and 172.42±3.21 *ppm* than FHm which ranged lower from 54.85±0.60, 54.85±0.60, 421.47±9.01 and 85.24±1.21 *ppm*, respectively. Further analysis of this trend of results showed that there was increasing trend of metal contamination with machine type where ArtHm scored higher metal contents than FHm as evidenced from samples in (**Table 4**) Serial no. 2, 3,4, 5, 6, 7, 8 and 9. As such this trend of results is insightful and presented suitable information in the choice of materials for beaters' fabrication. Furthermore, comparing Hm to with control treatment, it was shown that Hp achieved the lowest whose findings were comparable to maximum set limits (World Food Program, 2011, and FAO /WHO, 1983). In this trend of results by Hp, were therefore attributed to the fact that the its metal elements were only from those absorbed by the plant during its growth unlike by contamination by hammer milling. In the preceding section literature also confirmed that higher availability and accessibility of these metals Pb, Cd, Cu, As, still remain a major global threat in food chain (Somers, 1974, Cheftel, 1988, Normanyo *et al* 2010) just as supported by Järup, (2003) for Cd and Pb exposure which cause ill health to humans and that their concentrations need to be minimized to enhance food safety by standardizing flour it and making it comparable to Hp and recommended metal content limits. As mechanical determination of weight loss of the beaters due to milling was also important in the present study. **Figure. 6**, depicts gradual weight loss of beaters where the results showed that as milling of grain samples progressed, for sixty-six days, the gradual weight loss of the beaters also occurred concurrently and so was the metal contamination in flour. This explains the comparison between FHm with ArtHm, showed that FHm achieved less wearing rate than ArtHm treatment, comparing 60.48 and 75.02 *ppm*, respectively. These results explained why ArtHm achieved higher flour contamination than FHm. As such choice of materials for hammer mill beaters fabrication is crucial to effective hammer mill design for enhanced food safety.

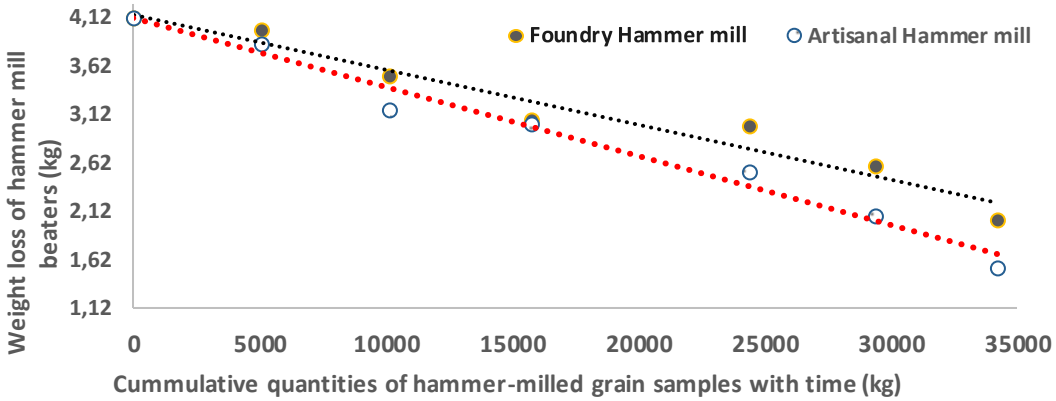


Fig.6. Gradual weight loss of beaters due to continued hammer milling of grain samples.

For the metal removal in flour by metal detection in Hp, FHM and ArtHm samples **Table 5**, depicts detected heavy metal presence in flour samples. The results showed that Hp showed no metal detected whereas all machine types had much higher metal presence confirming heavy metal contamination in flour.

Table 4: Metal content in wet weight mean ± SEM [ppm] with methods of flour production in Malawi.

Sample	FHm								Art Hm								Hp							
	Zn	Cu	Mn	Fe	Al	As	Pb	Cd	Zn	Cu	Mn	Fe	Al	As	Pb	Cd	Zn	Cu	Mn	Fe	Al	As	Pb	Cd
1	28.60± 0.80	0.60	±6.11	±6.21	0.90	0.60	±9.01	1.21	0.60	0.34	±4.11	382.32 ±8.21	0.40	0.44	±6.21	±3.21	19.18 ±4.0	0.26 ± 0.02	1.07 ± 0.90	8.23 ± 0.70	4.18 ± 4.0	0.36 ± 0.12	3.08 ± 0.12	0.07 ± 0.1
2	25.10±2. 11	45.16±5. 13	290.11± 6.12	163.30± 4.07	22.12±3. 11	34.06±7. 13	300.07± 7.02	102.32± 1.71	28.10±2. 11	54.26±7. 13	400.09± 6.02	183.37± 8.07	28.10±2. 11	53.27±7. 10	400.14± 8.02	112.32± 3.21	13.88 ± 3.0	0.47 ± 0.82	2.7 ± 0.42	7.43 ± 0.21	3.91 ± 3.00	0.57 ± 0.88	0.90±4.1 3	0.00
3	15.23± 1.50	34.49± 0.16	0.99±9. 31	98.44± 3.61	18.56± 4.63	28.23± 2.31	26.17± 8.30	72.61± 0.13	20.12± 3.10	50.36± 8.13	380.61 ±4.12	170.12 6±2.13	20.36± 6.13	45.60± 11.03	26.38± 4.23	91.26± 4.11	9.14 ± 1.1	1.48 ± 0.03	1.84 ± 0.10	43.3 ± 5.0	2.09±2. 10	0.43±9. 10	0.03±1. 03	0.34 ± 0.15
4	3.76±1. 21	12.78± 0.70	0.08±3. 12	70.23± 6.10	6±0.13	26.07± 4.13	16.22± 1.11	62±4.3 1	16.63± 4.15	20.26± 0.13	230.46 ±11.10	130.26 ±0.33	12.26± 0.43	23.20± 1.30	16.02± 0.10	60.03± 3.10	4.46 ± 6.00	0.14 ± 7.02	1.04 ± 0.02	24.04 ±4.23	1.09±1. 13	0.26±1. 34	0.026± 1.32	0.06±1. 11
5	28.6±0.8 0	58.65±0. 60	532.75± 0.11	365.27± 5.21	48.60±1. 80	58.95±2. 60	431.40± 2.01	872.02± 8.71	34.61±0. 61	77.85±0. 64	572.09± 7.11	385.38± 7.23	47.67±0. 39	82.85±4. 46	63.06±7. 21	187.41± 4.41	21.19 ± 6.0	0.29 ± 0.72	1.27 ± 0.11	20.22 ± 0.76	12.16 ± 6.02	0.86 ± 0.72	6.08 ± 0.19	0.09 ± 0.19
6	38.67±0 .99	74.89±0 .80	562.85± 5.11	372.29± 7.26	48.40±0 .49	60.65±0 .69	57.40±1 .21	892.49± 0.21	55.12±0 .66	78.95±0 .94	572.09± 3.13	392.42± 9.20	49.70±0 .49	79.55±0 .64	70.09±7 .87	182.29± 4.28	29.10 ± 7.0	2.26 ± 0.32	4.07 ± 0.13	28.22 ± 0.90	7.38 ± 8.0	6.76 ± 0.62	10.28 ± 1.82	1.09 ± 0.87
7	32.71±0 .49	64.81±0 .70	546.55± 7.19	356.27± 4.26	41.49±0 .19	590.57± 0.99	49.74±2 .24	798.94± 3.11	50.19±0 .64	68.55±0 .90	552.08± 4.18	371.12± 0.29	43.77±0 .69	69.55±0 .69	60.24±8 .77	173.09± 0.68	25.16 ± 8.0	1.06 ± 0.27	3.47 ± 0.19	23.28 ± 0.99	6.99 ± 9.09	4.16 ± 0.42	8.98 ± 0.52	3.074 ± 2.71
8	26.61±0. 87	34.98±0. 90	427.46± 8.91	300.29± 9.29	29.47±0. 75	64.71±0. 69	386.47± 9.01	559.32± 1.91	30.16±4. 10	64.88±1. 37	442.09± 7.10	342.72± 7.27	35.90±0. 89	58.59±0. 94	487.99± 8.81	144.92± 1.24	15.18 ± 8.0	0.20 ± 0.99	0.99 ± 0.94	6.93 ± 0.05	3.99 ± 3.94	1.34 ± 0.88	0.98 ± 3.92	0.97 ± 0.19
9	48.69± 0.70	74.81± 0.69	572.95 ±9.11	372.96 ±8.29	48.01± 4.90	61.89± 7.7	492.47 ±0.01	888.92 ±0.41	45.99± 0.68	84.88± 0.84	582.95 ±6.14	397.37 ±5.29	65.80± 0.49	75.59± 5.64	690.09 ±9.01	185.45 ±0.21	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a

1 denotes Whole maize grain, *Mgaiwa*, flour, 2 denotes Dehulled and unsoaked maize / Grain meal flour, 3 denotes Dehulled Soaked maize / white flour or *Ufa woyela*, 4 denotes Maize bran, *Gaga* or *madeya*, flour, 5 denotes Mixture of soybean and whole maize flour, 6 denotes Mixture of soybean, maize, sorghum, rice and groundnut flour, 7 denotes Mixture of soybean, maize, sorghum and groundnut flour, 8 denotes Soaked and fermented maize, *Chimera*, flour and 9 denoting Emergency sample of whole maize grain, *Maaiwa*, flour (*Note: Emergency flour sample denotes the flour sample that was collected*

Table 1: Heavy metal tracing in hand-pound and hammer-milled flour samples

	(a) Heavy Metal traces in Hp samples		**(b) Heavy Metal traces in FHm & Art Hm samples**	
1	Whole grain maize flour "*Mgaiwa*"	**no**	Whole maize grain, *Mgaiwa*, flour	**yes**
2	Dehulled Soaked maize / white flour "*Ufa woyera*"	**no**	Dehulled and unsoaked maize / Grain meal flour	**yes**
3	Dehulled unsoaked maize / Grain meal flour	**no**	Dehulled Soaked maize / white *Ufa woyela*, flour	**yes**
4	Maize bran, *Gaga* or *madeya*, flour	**no**	Maize bran, *Gaga* or *madeya*, flour	**yes**
5	Soaked and fermented maize, *Chimera*, flour	**no**	Mixture of soybean and maize flour	**yes**
6	Mixture of soybean and maize flour	**no**	Mixture of soybean, maize, sorghum rice and groundnut flour	**yes**
7	Mixture of soybean, maize, sorghum, rice and groundnut flour	**no**	Mixture of soybean, maize, sorghum and groundnut flour	**yes**
8	Mixture of soybean, maize, sorghum and groundnut flour	**no**	Soaked and fermented maize, *Chimera*, flour	**yes**
9			Emergency sample of whole maize grain, flour	**yes,**

Emergency flour sample denotes the flour sample that was collected incidentally when some metallic object fell into the

Conclusions and Recommendations

Food security, in the current generation is heavily threatened by the abundant presence of heavy metal poisoning of food and is worrisome in the SADC region. The use of crudely designed hammer-mills is one major causes of heavy metal contamination in the flour samples. In the present study, heavy metal contaminants in Hammer-milled flour compared to hand-pounding was successfully and mechanically quantified, in which significant heavy metal contamination in food chain was confirmed to be significant. In conclusion, for enhanced food safety, there is urgent need reduce the metal-contaminants and standardize the flour quality to the people. As such it is now time for policy makers to standardize the flour quality to protect mankind from this threat of food safety. Based these findings devisable mechanical concepts for hammer mill redesign to minimize the heavy metal contents in food products is recommended. This design strategy would be instrumental to improve flour quality for the respective in SADCC region Bureau of Standards for both Malawi (2014) and Mozambique and ensure for optimum removal of heavy metal contaminants such mechanical concepts as proposed by Normanyo *et al*, (2010) and CAC/RCP, (1969) would be recommended. Their concepts incorporate magnetic metal separator and detectors in line with belt conveyor (Helmich, 2014).

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HYBRID SIEVE-HEATER AND INFRARED DRYING OF SAFFRON STIGMA: EFFECTS OF TEMPERATURE, AIR VELOCITY AND INFRARED RADIATION ON KINETICS AND QUALITY PARAMETERS

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Abstract

Saffron is a dry, Red color stigma and stylets of the *Crocus sativus L.* which is the most expensive spice in the world. Drying is an important step in the process of saffron production. It causes physical, chemical and biochemical changes to achieve the desired properties of saffron. In this study, the kinetics of drying saffron stigma using a novel method with Hybrid Sieve-Heater and infrared dryer at two temperature levels of 40, 45 °C, two hot air flow speeds of 0.3 and 0.6 m/s and two levels of 125 W and 250 W radiation were studied. An image-based method for measuring tortuosity of saffron stigma was also proposed. The tortuosity of saffron could be an indicator for its shrinkage. The effects of the studied parameters on the amount of Crocin, Picocrocin and Safranal and of the final product were also investigated. The results showed that, the two term model was found to be the best model in fitting data of both drying methods. Increased lamp power, temperature and air velocity caused reduction in the drying time of saffron. The lowest amount of tortuosity variation was related to the traditional drying method in the shade and treatment at 40 ° C, hot air speed 0.3 m/s, and infrared radiation of 125 watts. In terms of maintaining the color strength of saffron, the highest Crocin was obtained when the Hybrid Sieve-Heater and infrared dryer was adjusted at 40 ° C, hot air speed 0.6 m/s, and infrared radiation of 125 watts which was the highest among all other treatments.

Keywords: *Saffron, Hybrid drying, air velocity, kinetics, quality*

Introduction

Saffron, a spice produced from the red stigmas of the flowers of *Crocus sativus* L. is an added-value agricultural product due to its coloring, flavoring and biological properties (Carmona *et al.*, 2005). Iran is the largest saffron producer in the world. 94% of global saffron production belongs to Iran. The main active ingredients of saffron are Crostein and Crocin esters, which are responsible for its color strength. Moreover, the quality of saffron depends also on several factors such as agricultural practices, environment, post-harvest processing and storage conditions which are crucial factors in determining the quality of the spice. Drying is an important step in the saffron processing. It causes the physical, chemical and biochemical changes required to achieve the desired properties of saffron (Husaini *et al.*, 2010).

Generally, in Iran drying is carried out under shade which usually takes 27-53 hours to dry the product to a safe moisture level of 8%. Slow drying results in quality deterioration of saffron. In Spain, stigmas are placed on a sieve with a silk bottom that is placed over the heating source, which can be a gas cooker, live vine-shoot charcoal or, to a lesser extent, an electric coil (Carmona *et al.*, 2005).

New drying methods have been developed and introduced recently, one of which is infrared drying. The IR heating presents some advantages such as decreasing drying time, high-energy

efficiency and lower environmental impact as well as improved product quality (Celma *et al.*, 2009). The combination of infrared and hot air drying compared to the hot air drying employs lower air temperature and can be a suitable alternative for heat-sensitive products .In addition infrared drying can be used in rural areas for low capacity and low cost applications (Pan and Atungulu, 2010).

The thermal degradation of heat-sensitive products is far lower than that of hot air drying (Nadian *et al.*, 2016). The product temperature is a crucial parameter affecting not only duration of the drying process, but also colour changes and shrinkage. Most of saffron in Iran is dried up by farmers.

The main objectives of this study was to determine and test the most appropriate thin layer drying model and comparing Crocin, Picrocrocin, Safranal and tortuosity on different treatments.

Materials and methods

Saffron was picked before sunlight from a farm around Gonabad (34.3396° N, 58.7030° E, Iran) at November 2017. Stigmas were separated from the other parts of the flower. The flowers on each experimental plot were picked by hand at approximately the same time of day.

Experimental Setup

The hybrid Sieve-Heater and infrared dryer (SHD) used in this study was developed in the department of Food Science and Technology Ferdowsi University of Mashhad in Iran.The device consisted of two thermal elements, a fan and an infrared lamp holder. The hot air encounters a cylindrical chamber in diameter of 32 cm and a height of 50 cm from the bottom to the Sieve. A PT100, Thermal Resistance Temperature Sensor (Shanghai Feilong Meters & Electronics Co., Ltd., China) was installed and used to control the temperature of the hot air. A contactor was also used to illuminate the infrared lamp, and another contactor was needed to turn on two 250 watt electric coils. Moreover, one 250 W IR lamp and one 125 W IR lamp (NOOR, NIR, IRAN). The drying temperature is manually adjusted and the microcontroller was planned.

Drying Experiments

Two levels of 125 W and 250 W radiation, two levels of 40 and 45 C, and two speeds of hot air flow of 0.3 and 0.6 m/s were used for dehydration process. The time to reach moisture content of 8% (based on wet weight) was considered as the end point of the drying process. Also, the samples were dried with a sieve-heater dryer (Spanish method). The samples were also dried in shade (the traditional dehydration process in Gonabad, Iran).

Modeling of drying kinetics

Moisture ratio during thin layer drying of saffron was calculated as follows:

$$MR = \frac{M_t - M_e}{M_0 - M_e} \qquad (1)$$

MR: Moisture ratio (dimensionless), M_t: Moisture of the samples at any moment based on dryness (d.b.) *Me*: Equilibrium moisture content of the samples (d.b.) M_0: Primary moisture content of the samples (d.b.). Equilibrium moisture content (*Me*) of samples in each temperature and relative humidity is relatively small compared to M_0:, Therefore it can be simplified as follows:

$$MR = \frac{M_t}{M_0} \qquad (2)$$

Drying curves were fitted to ten different moisture ratio models.

Image acquisition

Image acquisition was carried out using a mobile phone camera (Samsung Galaxy S7 Edge SM-G935FD Dual SIM 32GB Mobile Phone) which was placed on an imaging chamber at a distance of 9 cm from the sample. The shutter speed was 1/500 s without employing flash and respectively lens focal length, Diaphragm value and ISO was 4/2 mm, F1/7 and 100. Images were captured at their maximum resolution (3024×4032 pixels) and were saved in ''.JPG'' format.

Image Pre-Processing

The images were converted from RGB to the gray-scale and then the global image threshold was achieved using Otsu's method.

Measurement of tortuosity based on image processing

For quantitative measurement of tortuosity, the stigma was modeled as a smooth connected curve.

The method employed in this study was a curvature-based tortuosity measurement. To calculate curvature, a new approach called the template disk method is used (Aghamohamadian-Sharbaf *et al.*, 2016).

for a plan curve y = f(x), curvature is given by

$$\kappa = \frac{y''}{(1+y'^2)^{\frac{3}{2}}} \quad (3)$$

For a plane curve given parametrically in Cartesian coordinates as x=x(t) and y=y(t), the curvature is:

$$\kappa = \frac{|x'y'' - y'x''|}{\left(x'^2 + y'^2\right)^{\frac{3}{2}}} \quad (4)$$

Given the fact that numerical methods are time consuming, the template disc method for 2D images was used. After simplifying the functions and formulas the nonlinear estimation of curvature κ_{nl} is defined as:

$$\kappa_{nl} \triangleq A_c \quad (5)$$

Based on the one method of local tortuosity calculation, global tortuosity measures are called τ_{nl} (Aghamohamadian-Sharbaf *et al.*, 2016).

$$\tau = \frac{1}{m}\sum_{i=1}^{m} \kappa_i \quad (6)$$

Determine the saffron smoothness according to expert opinion

Considering that national and international standards do not define the smoothness of saffron stigma, therefore, experts' experiences were considered. Four experts who were buying and selling saffron in the Gonabad local market in Iran were selected. The five degrees of quality for saffron stigma was considered (Very bad, bad, average, good, very good). In addition, the amount of smoothness is the opposite of tortuosity.

Determination of the main saffron characteristics using UV–vis spectrometric method

Ultraviolet–visible (UV–vis) spectra were recorded using a Shimadzu spectrophotometer (model 1600 PC) in the range from 700 to 200 nm using quartz cell (1 cm path-length). Analyses were always carried out in triplicate. Absorbance readings at 257, 330 and 440 were related back to the 1% solution and expressed as $E_{1\%}$ (257 nm), $E_{1\%}$ (330 nm) and $E_{1\%}$ (440 nm), according to the ISO 3632 for the standardized measurement of Picrocrocin, Safranal and Crocin (coloring strength), respectively. All analyses were carried out in triplicate (Minaei *et al.*, 2017) & (Jafari *et al.*, 2018).

Statistical Analysis

Data were analyzed by Analysis of Variance (ANOVA) applying Duncan's test at 95% of confidence level as a post-hoc test (IBM SPSS st.23). Experimental drying data were fitted using nonlinear regression performed through MATLAB software (2017b. ver 9.3).

Results and discussion

Drying kinetics modeling

The coefficient of determination (R^2) is considered as one of the prime criteria for the selection of the best model (Taylor, 1997). Furthermore, the evaluation of the goodness of fit of the model was also determined by various widely used statistical parameters, such as square error ($\chi 2$) and root mean square error (RMSE). For the quality fit, R^2 value should be higher; while $\chi 2$ and RMSE value should be lower (Ambros *et al.*, 2018).

With regard to the lesser error due to the fitting of laboratory data during the drying of saffron, the Two-term model was selected that the results of this model were reported in Table 1.

Table 1 Fixed values and Two-term model coefficients differentiated by drying temperature and hot air

Temperature (°C)	Velocity (m/s)	IR lamp Power (watt)	K_0	a	b	n	K_1	R^2	RMSE	$\chi 2$
40	0.3	125	0.1211	-0.2257	1.226	0.6871	0.05779	0.9996	0.007955	**3.45E-05**
40	0.3	250	0.00014	0.2402	0.763	2.724	0.09282	0.9997	0.007778	**2.69E-05**
40	0.6	125	0.008229	0.825	0.175	1.489	5.222	0.9996	0.008132	**3.68E-05**
40	0.6	250	0.000121	0.4299	0.5724	2.905	0.1231	0.9995	0.01152	**4.98E-05**
45	0.3	125	0.0142	0.5564	0.4439	1.586	0.1529	0.9999	0.003511	**5.88E-06**
45	0.3	250	0.003488	0.5176	0.4815	2.124	0.2293	0.9998	0.005465	**1.09E-05**
45	0.6	125	0.06708	1.001	-0.00213	1.106	-0.12	0.9997	0.00645	**1.72E-05**
45	0.6	250	0.00356	0.5545	0.444	2.119	0.287	0.9996	0.007328	**1.35E-05**

The effect of type and temperature of drying on the drying time

The temperature of hot air and Infra-Red radiation, as well as their interaction, were significant ($p<0.05$) on drying time of saffron. The shortest drying time occurred at 45 ° C and infrared radiation of 250 watts. At the temperature of 40 °C by increasing the lamp power from 125 watts to 250 watts and increasing the air flow rate from 0.3 to 0.6 m/s the drying time decreased 1.5 times. Also At the temperature of 45 °C by increasing the lamp power from 125 watts to 250 watts and increasing the air flow rate from 0.3 to 0.6 m/s the drying time decreased 1.4 times. By increasing the drying temperature from 40 to 45, the drying time

decreased by 1.5 to 2 times (Dependent on lamp power and air flow velocity). The drying time was reduced by nearly 48% in Combined hot air-infrared dryer (for potatoes and carrots) compared to hot air drying (Hebbar *et al.*, 2004). Sui and et al. (2014) reported that Infrared drying had the highest drying rate, which reduced the drying time by more than 47.3% compared with other methods (Sui *et al.*, 2014). Sharma et al. (2005) Stated that in thin layer drying of onion slices, using infrared-convective dryer when the infrared lamp power was changed from 300 to 500 watts and the air temperature increased from 35 to 45° C and the air flow rate increased from 1 to 1.5 m/s, drying time reduced by 2.5 times (Sharma *et al.*, 2005). At constant temperatures, increasing the power or intensity of infrared light and reducing the air speed shortens the drying time. Increasing the lamp power and, consequently, increasing the intensity of the infrared radiation, increases the infrared energy penetration into the product. As a result, the drying time is reduced (Pan and Atungulu, 2010).

The effect of drying methods on stigmas tortuosity

Stigmas tortuosity variation results are presented in the Table 2. The results showed that drying methods had significantly different effects on stigmas tortuosity ($p<0.05$). The lowest amount of $\Delta\tau_{nl}$ (tortuosity variation) was related to the traditional drying method in the shade. However, there was no significant difference between this drying method and drying treatment at 40 ° C, hot air speed 0.3 m/s, and infrared radiation of 125 watts. On the other hand, the highest amount of $\Delta\tau_{nl}$ was related to the Sieve-Heater drying (Spanish method). According to the results, the temperature increase from 40 ° C to 45 ° C, increases the tortuosity. Drying with Sieve-Heater is a hot air drying method. Due to the fact that drying with hot air from the surface begins to dry, the degree of shrinkage and hardening of the surface is more than the combined method. Quality indicators such as color and surface hardening and shrinkage of different products, such as potatoes and carrots, have been better with combined drying methods than drying them alone with infrared or hot air. It is also suggested that drying hybrid methods be used for heat-sensitive products (Pan and Atungulu, 2010) & (Karam *et al.*, 2016). The results of analysis of variance of expert scoring for different treatments confirmed these reported results ($p <0.05$). The highest scores of stigmas smoothness were given to dried shade and drying treatment at 40 ° C, hot air speed 0.3 m/s, and infrared radiation of 125 watts (table 4). Suggested algorithm for measurement of tortuosity is simplicity of the measure, low-computational burden and The running time for measure is nearly 35 s (Aghamohamadian-Sharbaf *et al.*, 2016). This method can also be used to calculate the tortuosity of saffron stigma during the drying period.

Table 2 Comparison between Shave, Sieve-Heater and hybrid Sieve-Heater and infrared drying methods on tortuosity Changes

Temperature (°C)	Velocity (m/s)	IR lamp Power (watt)	τ_{nl}	$\Delta\tau_{nl}$
40	**0.3**	**250**	**1.064593664 ± 0.009245423**	**0.0395937[b]**
40	**0.6**	**250**	**1.056908574 ± 0.014357261**	**0.0319086[b]**
40	**0.3**	**125**	**1.044143836 ± 0.014903012**	**0.0156438[a]**
40	**0.6**	**125**	**1.066759414 ± 0.018920055**	**0.0382594[b]**
45	**0.3**	**250**	**1.067114119 ± 0.005869891**	**0.0428141[b]**
45	**0.6**	**250**	**1.078490187 ± 0.031523263**	**0.0539902[b]**
45	**0.3**	**125**	**1.066009214 ± 0.002702378**	**0.0375092b**
45	**0.6**	**125**	**1.076853685 ± 0.012877911**	**0.0483537[b]**

Shave	**1.03255789 ± 0.004121658**	**0.0075579[a]**
Sieve-Heater	**1.0899 ± 0.012707872**	**0.0649000[b]**
Fresh	**1.02628 ± 0.001858**	**_____**

Table 3 Average scores of experts for saffron smoothness by direct seeing samples and images

Temperature (°C)	Velocity (m/s)	IR lamp Power (watt)	Average scores of experts by direct seeing samples	Average scores of experts by seeing images
40	**0.3**	**250**	**3.0833[d]**	**3.9167[ab]**
40	**0.6**	**250**	**3.1667[c]**	**3.5833[bc]**
40	**0.3**	**125**	**3.8333[ab]**	**4.2500[a]**
40	**0.6**	**125**	**3.3333[c]**	**3.5000[bc]**
45	**0.3**	**250**	**3.0833[d]**	**3.9167[a]**
45	**0.6**	**250**	**3.1667[c]**	**3.5833[bc]**
45	**0.3**	**125**	**3.5000[bc]**	**3.6667[abc]**
45	**0.6**	**125**	**3.2500[c]**	**3.6667[abc]**
	Shave		**3.9167[a]**	**4.0833[a]**
	Sieve-Heater		**3.2500[c]**	**3.2500[c]**

The effect of type and temperature of drying on Crocin, Safranal and Picrocrocin

Maintaining the quality of food and dried crops is one of the most important indicators of the success of drying systems. The quality of saffron depends on the concentration of its major metabolites (Lage and Cantrell, 2009). Drying methods that can keep these metabolites higher will be more desirable.

The Crocin, Safranal and Picrocrocin contents of the samples dried naturally in shade and using the sieve-heater dryer and the SHD were analyzed by a spectrophotometer. The type of drying and temperature method had a significant effect on Crocin and Picrocrocin ($p < 0.05$) but there was no effect on Safranal. The highest amount of Crocin was related to drying at 40 ° C, hot air speed 0.6 m/s, and infrared radiation of 125 watts, and the least amount was in the traditional way (Shade) and sieve-heater drying (Fig. 1A). The highest amount of Picrocrocin was obtained for drying at 45 ° C, hot air speed 0.3 m/s, and infrared radiation of 125 watts, and the lowest amount was drying in the shade (Fig. 1B). Mortezapour et al. (2012) examined the quality properties of saffron in a Heat Pump Assisted Hybrid Photovoltaic-thermal Solar Dryer at three temperatures of 40, 50 and 60° C.

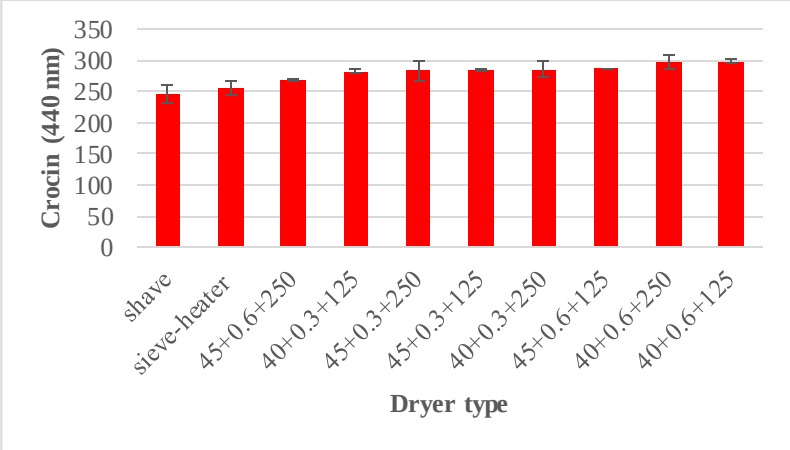


Fig 1A

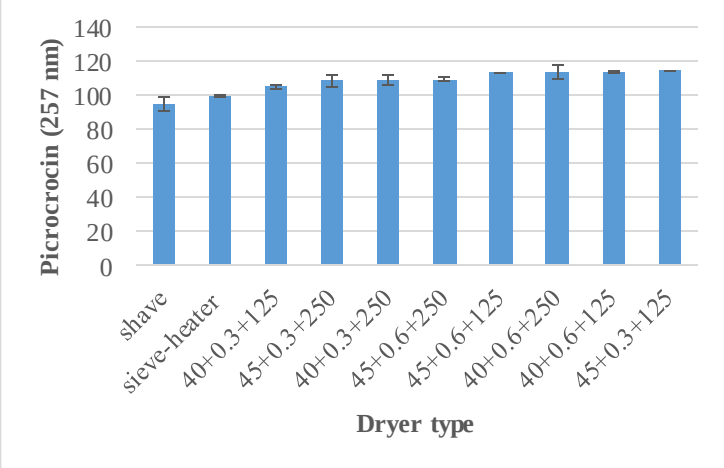


Fig 1B

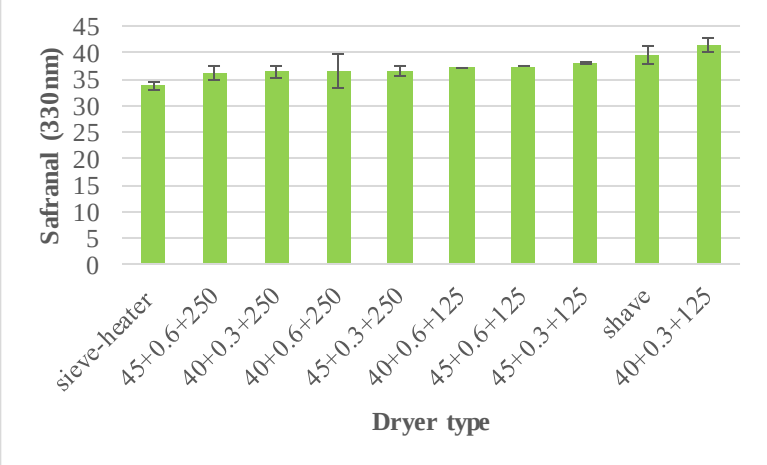


Fig 1C

Figure 1 Changes of Crocin (A), Picrocrocin (B) and Safranal (C)

The results of drying saffron quality evaluation showed that increasing the temperature of the drying air and the heat pump improved the color of saffron (Crocin) and the increase in temperature reduced the aroma of saffron (Safranal). However, temperature changes and drying methods could not significantly change the taste of saffron (Picocrocin) (Mortezapour *et al.*, 2012). Carmona et al. (2005) examined three drying treatments for saffron: drying at room temperature, drying with hot air at various temperatures (70, 90 and 110 ° C) and drying

in accordance with the traditional methods of the Castille-La Mancha (Spain) with three different heat sources (coal boiler, gas cooker and electric element). The time was between 28 and 55 minutes and the average temperature was between 54 and 83 degrees Celsius for the traditional Spanish drying method. The highest color strength (Crocin) was obtained when the saffron was processed at high temperature and low time (Carmona *et al.*, 2005). Del Campo et al. (2010) investigated the effects three temperature conditions of 18 to 20 ° C for 24 hours and 40 to 50 ° C for 75 minutes and 55 ° C for 75 minutes on quality parameters of saffron including Crocin, Picrocrocin and Safranal. The highest Crocin was related to drying at 40 to 50 ° C, but there was no significant difference with drying at 55 ° C. (Del Campo *et al.*, 2010). Using an oven at 40 °C increases the amount of Crocin significantly by comparing traditional methods (Chaouqi *et al.*, 2018). The results of the above studies confirm our results. Although there was no statistically significant difference in the amount of Safranal between treatments ($p>0.05$) but in terms of number of Safranal, two treatments were higher: drying at 40 ° C, hot air speed 0.6 m/s, and infrared radiation of 125 watts and drying at shade (Fig. 1C). Hosaini et al. (2010) stated that the drying of saffron in vacuum and cross-flow drying would significantly reduce the amount of Safranal**.** treatments that were dried in shade or under rapid conditions have a better taste profile that was associated with higher Safranal levels (Husaini *et al.*, 2010). Gregory et al. (2005) examined the effect of drying temperature and air flow on secondary metabolites of saffron. The results showed that the amount of Safranal in a method that was first heated for 20 minutes at a temperature of 80 to 90 ° C and then dried at 43 ° C was 25 times higher than the saffron dried at low temperature. They also reported that the air flow reduced the amount of Safranal (Gregory *et al.*, 2005). We used low air velocity to dry the saffron, which was effective in protecting Safranal.

Conclusions

A hybrid Sieve-Heater and infrared drying was developed for saffron stigmas. According to the statistical indices R2, $\chi 2$ and RMSE, the two term model is better for the kinetics and prediction of the drying process of saffron stigma at two temperatures of 40 and 45 °C. By increasing the drying temperature from 40 to 45 and increasing the lamp power and air velocity, the drying time decreased by 1.5 to 2 times. A new method was introduced for determining the tortuosity of saffron stigma which could be an indicator for the shrinkage of saffron. This algorithm is a curvature-based algorithm. For curvature calculation, the template disk method was used. The results of this algorithm were consistent with the sensory evaluation of the experts. Drying in the shade, as well as a temperature of 40 ° C, hot air velocity 0.3 m/s, and infrared radiation of 125 watts, produced the lowest tortuosity. The hybrid Sieve-Heater and infrared drying had a significant effect on preserving the Crocin, Safranal and Picrocrocin of saffron.

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ORGANIC AGRICULTURE

CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF BLACKBERRY, BLACK CHOKEBERRY AND JOSTABERRY

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Abstract

There is a general belief that the brighter and more deeply colored fruits contain higher levels of antioxidants, minerals and vitamins. Three black-fruited bushes: blackberry (*Rubus fruticosus* 'Thornfree'), black chokeberry (*Aronia* melanocarpa 'Nero') and jostaberry (*Ribes* x *nidigrolaria* `Josta`) were grown under identical agro-ecological conditions, practicing organic farming without chemicals. The fruits of this species were analyzed for nutritional properties, essential elements, bioactive content and antioxidant potentials. Dry weight, total ash, acidity, total sugar and vitamin C were determined according to AOAC. Mineral elements (P, K, Ca, Mg, Fe, Mn, Zn, Cu, Na, Al, Se, Cd, Cr, Pb) were quantified by flame atomic absorption spectrometry. Total flavonoids were determined by Kumaran and the total flavonols by Ordonez method. Anthocyanins were quantificated spectrophotometrically by modified single pH and pH differential method, described by Sun, Chu, Wu & Liu. Total phenolic content was determined using Folin-Ciocalteu reagent assay. Antioxidant activity of fruits has been evaluated by ABTS, DPPH and FRAP assays, in the manner described by Pellegrini *et al.*, Liyana-Pathiranana & Shahidi and Benzie & Strain, respectively.
All three species have a fairly uniform elemental composition. Only black chokeberry has a significantly higher zinc content (2.07 mg/100 g). On the other hand, jostaberry stand out in acidity (2.4 %) and vitamin C concentration (78.3 mg/100 g). When it comes to phytochemicals, black chokeberry dominates superiorly in the content of anthocyanins (45.92 μg/mg), flavonols (0.77 mgQcE/g), flavonoids (71.42 mgQcE/g) and phenols (74.80 mg GAE /g). The same is with the antioxidant capacity for all three assays: 0.17 mg Trolox/mL (ABTS), 0.97 mg Trolox/mL (DPPH) and 0.10 mmol Fe (II)/mg $_{F.W.}$ (FRAP).
The presented results affirm three black-fruited bushes as valuable and healthy fruit crops, interesting not only for commercial cultivation, but also for peri-urban farming and urban gardening.

Key words: *blackberry, black chokeberry, jostaberry, antioxidant properties, phytochemicals*

Introduction

Small fruits are popular residents of home gardens. In principle, they require less space and give a higher yield per unit area compared to fruit trees. Also, they are easier to harvest, prune, maintain and protect. In addition, they are distinguished by a high content of physiologically active substances and antioxidant properties. In that respect, blackberry, black chokebbery and jostaberry are particularly prominent. Wild growing blackberries are widespread in most of Europe, Western and Central Asia, North Africa and the North America. They grow along the edges of forests, forming stands of shrubs, thickets and hedges, mostly on dry and warm soils, from lowlands to hilly areas. Edible fruits are delicious and juicy. They are eaten raw or processed into juices, jams, etc. Leaves are also usable, fresh, dried or fermented. In the nineties of the last century, thornless cultivars arrived in our region.

Today, in domestic commercial plantations, the following cultivars can be found: `Apache`, `Chester Thornless`, `Čačanska bestrna` `Loch Ness`, `Thornfree`, `Triple Crown`, of which the most represented are: `Chester Thornless`, `Čačanska bestrna`and `Thornfree`. Thorny cultivars, such as `Darrow`,`Gazda` and `Illini Hardi` have not found wider application. Chokeberry (*Aronia* sp.) is an allochthonous species, native to North America, which was introduced to our region about ten years ago. In fact, since the beginning of the 20th century, it has been planted individually as a decorative horticultural species, while its main use values were unknown at that time. Taxon *Aronia rotundifolia* (Lam.) Pers. is mentioned as wild species in the Western Balkans and Central Europe, and that as a synonym for the species *Amelanchier ovalis* Medik. (Hartinger & Dalla Torre, 1882; Jovanović, 1958). Grlić (1980) thinks about wild chokeberry in a similar way. Jakobek *et al*. (2012) even analyzed the chemical composition of wild chokeberries in Croatia, but failed to state the scientific name of this species. Today, three taxa can be found in domestic commercial plantations: black chockeberry (*Aronia melanocarpa* (Michx.) Elliott, red chokeberry (*Aronia arbutifolia* (L.) Pers. and purple chokeberry (*Aroni*a ×*prunifolia* (Marshall.) Rehder, which is a polyploid hybrid of *A. melanocarpa* and *A. arbutifolia*. The most common taxon is black chockeberry with cultivars `Nero` and` Viking`. Of the same species, one can still find cultivars: `Aron`,`Hugin`and `Rubina`, and very rarely 'Fertödi '(from Hungary)` Galicjanka`, (from Poland) and `Moskva` (from Russia). The fruit of chokeberry is used fresh, dried in the form of tea, then as a juice, wine and jam. Dried and pulverized fruit is used as an addition to bread, improving its flavor and taste, as well as its surface appearance (Petković et al., 2019). Also, chokeberry is attributed to a wide range of medicinal properties, as evidenced by numerous scientific papers. Only in the field of prevention of chronic diseases, Jurikova and associates (2017) cite 74 published papers in the period from 2012 to 2016, or 145 papers on this topic from 1997 to 2017. As in the case of blackberries, chokeberry leaf also has a powerful medicinal and therapeutic properties (Shahin et al., 2019). Josta (*Ribes* x *nidigrolaria* Rud. Bauer & A. Bauer.) is an artificially designed taxon (so-called cultigen). It was created by Dr. Rudolf Bauer from West Germany in 1977 as a complex F_2 hybrid between a black currant cultivar (*Ribes nigrum* 'Langtraubige Schwarze') and a F_1 hybrid of another black currant cultivar (*Ribes nigurm* 'Silvergieters Schwarze') and a gooseberry cultivar (*Ribes grossularia* 'Grune Hansa'); (Bauer R., 1978). The goal of hybridization was to produce a thornless plant with fruits the size of a gooseberry and the taste of black currant. Equally important were the protective moments. Thus, the cultivar *Ribes nigrum* 'Langtraubige Schwarze' was chosen because it is resistant to mildew (*Sphaerotheca mors-uvae* (Schw.)), and the F_1 hybrid because it is resistant to white pine blister rust (*Cronartium ribicola* J.C. Fisch). Ten years later Bauer A. (1986) cites a somewhat different formulation of jostaberry as a complex cross of (*Ribes nigrum* × *R. grossularia*) × (*R. nigrum* × *R. divaricatum*), where *R. divaricatum* is the North American coastal black gooseberry. Although jostaberry was introduced to Bosnia and Herzegovina soon after its creation, there are no intensive commercial josta plantations in B&H today[1/]. Therefore, there are no conditions for its escape to free nature, where it can otherwise be found in some countries and regions (Anon., 2011). In our country, jostaberry is mostly found on small, non-intensive plantations and in home gardens. No nursery in B&H produces certified seedlings of jostaberry. At the international level, in addition to the original cultivar `Josta`, there are also well known cultivars` Jostine` and `Jogranda` (mentioned as 'Jostagranda' and 'Jostaki` as well), then several cultivars grown in the Netherlands: `Anita`, `Caseille`, `Jonova`,` Rita` and in Switzerland: `Original Swiss Black` and` Swiss Red`. Also known are Swedish cultivar 'Kroma' [(*R. nigrum* × *R. grossularia*) × (*R. nigrum* × *R. niveum*)] and Russian hybrids 3231 and V 1323/3 (Podwyszynska & Pluta, 2019); in the USA there are cultivars: `Jostagranda`,` Jostina`, `Red Josta` (Josiah & Lackey, 2014). The fruit of jostberry are round-oval, reddish

to black. Their taste is sour and refreshing. They can be eaten fresh or processed into juice, jams, wine or vinegar. They are also used cooked in cakes and pies and added as a delicious flavor to homemade ice cream (Lim, 2012). The same author states that jostaberry exhibits significant antioxidant and antimicrobial activity. It is recommended for maintaining health, especially in anemia and for prevention of gastrointestinal and cardiovascular diseases (Kalugina I. & Kalugina J., 2017; Khanal *et al*., 2011). The aim of this paper is to analyze the chemical composition and antioxidant properties of these three small dark fruits, and to compare the obtained results with data from the same or related taxonomic units in researched and other areas.

Material and method

Material

All studied species were grown in the same urban garden in the city of Banja Luka, Bosnia and Herzegovina. The garden is owned and maintained by the NGO "The Society for the Environment and Sustainability on Earth – SENSE". It is agricultural land on alluvial gravelly sediments, with good water permeability and limited transmissibility. The land is flat, at an altitude of about 163 m. The average annual temperature is 10.8 ^{o}C, and for the vegetation period (from March to November) 17.2 ^{o}C. The average annual rainfall is 1,017 mm/m^2. Precipitation increases in spring and autumn, and decreases over summer and winter. The origin of the planting material is as follows: thornfree blackberry were purchased from the "Dana" nursery, situated in Romanovci near Banja Luka. Chokeberry were purchased from the "Vučković" nursery, located in Omarska, between Banja Luka and Prijedor. Josta were procured through the "Kmetijski inštitut Slovenije". Aronia and blackberry are arranged as a trellis system oriented in a north-south direction, while Josta is grown as unanchored island beds. Care and maintenance measures are carried out without the use of chemicals. The strip around the plants, 50 cm wide, is weeded and dug up by hand. Soil between the rows is not plowed, but only regularly mowed with a trimmer. The trellis is secured with concrete pillars. Three rows of steel wire are placed on each side of the pillars. In this way, the tying of shoots is avoided, their upright position is ensured and protection is provided in case of strong wind. Such solution also provides support against fractures in the event of late spring or early autumn snow.

/1/ The first josta seedlings (*Ribes* x *nidigrolaria* `Josta`) were brought to B&H in 1989 by Mr. Bruno Anić, a forestry engineer, employed in RO ŠIPAD "Jahorina" Sarajevo, responsible for the development of the non-wood forest products. The seedlings were obtained through the "Inštitut za sadjarstvo" Maribor (Slovenia) and planted at several sites in open forest areas around the city of Sarajevo (personal documentation of the author).

Method

Fruits were picked in the summer 2018 in the stage of full ripeness. The maturity degree was evaluated according to color and firmness. From the homogenised material, 5 g of samples was extracted with 80 % ethanol, twice at 25 ml. The solutions were refilled with 80 % ethanol up to 50 mL. Thus, 100 mg/mL concentrations were obtained and further used to determine total phenols, flavonoids, flavonols, neutralization of 2.2-diphenyl-1-picrylhydrazyl radical (DPPH) and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$) radicals. To determine total and monomeric anthocyanins, 20 g of samples was extracted with a 20 mL solution (85 ml of 95 % ethanol solution in 15 ml of 1.5 mol/L HCl solution) at 0 ^{o}C for 24 hours. After being left to stand, resulting mixture was filtered through a filter paper, and filtrates were used for further analysis. Total phenol content was determined by the modified Folin-Ciocalteu method (Wolfe & Liu, 2003). Gallic acid was used as a standard compound and the results were expressed as a phenol equivalent to the

gallic acid (GAE), i.e. μg GAE/mg. The total flavonoids are determined by the method of Kumaran & Karunakaran (2007), and the total flavonols according to the method of Ordoñez *et al*. (2006). Quercetin was used as the standard compound, and the results were expressed as μgQcE/mg. Total and monomeric anthocyanins were determined by the spectrophotometrically modified "single" pH and by the pH differential method (Sun, 2002). The antioxidant activity in relation to the DPPH radical was determined by the method of Liyana-Pathirana and Shahidi (2005). The modified method of Re *et al*. (1999) was used for the ABTS radical. The results were presented with the TEAC value (Trolox equivalent of antioxidant activity), i.e. as mgTrolox/mL. IC_{50} value (inhibitory concentration), i.e. efficient concentration of antioxidant necessary to decrease the initial radicals concentration by 50 % was determined from the plotted graph of scavenging activity against various concentrations of extracts. The lowest EC_{50} indicates the strongest ability of the extracts to act as a radical scavenger. Also, the antioxidant capacity of plant material was estimated following the FRAP procedure of Benzie & Strain (1996). The FRAP method is based on the ability of phenolic substances, dissolved in water, to reduce Fe^{3+} to Fe^{2+} ions. The resulting Fe^{2+} ions with TPTZ reagent (2,4,6-tripidyl-s-triazine) forms a blue-colored complex in an acidic medium with maximum absorption at 593 nm. Dry weight, total ash, acidity, total sugar and vitamin C was determined by the standard AOAC methods (Horwitz & AOAC, 2000). For the mineral content analysis, plant samples were homogenised in stainless steel rotating knife homogenizer (Tecator 1094, Foss, Hillerod, Denmark). All samples (0.5 g) were digested in duplicate using microwave digestion system, in accordance with the NF EN 13805 standard "Food stuffs - Determination of trace elements - Pressure digestion" and transfered into the volumetric flasks of 25.00 mL. ICP OES measurements were performed three times for each duplicate using Optima 8000 Optical Emission Spectrophotometer (Perkin Elmer, USA). The sample solutions were pumped by a peristaltic pump from tubes arranged on a Perkin Elmer auto-sampler model 510. For the calibration of the instrument, a certified standard solution CRM Instrument Calibration standard 2 was used, with a minerals concentration of 100 mg/L (Pb, Cd, As, Co, Ni, Cr, Se, Cu, Zn, Fe, Ca, Na, Mg, P , K, Mn, Mo) (Perkin Elmer, USA, LOT CL12-111YPY 1). The concentrations of the elements for the calibration direction were different. They were determined according to preliminary measurements for the determined metals.

Results

The results of the chemical analysis are shown in tables 1-3. When it comes to the nutritional properties of fresh fruits (table 1), among the observed species, black chokeberry has the highest content of dry matter and ash. It also has the highest pH value and the highest coefficient of sweetness. At the same time, jostaberry contains the most sugar and vitamin C, and has the highest acidity. Blackberry does not stand out in terms of any of the observed parameters.

Table 1: Nutritional properties of fresh fruits

Parameters	Unit	Blackberry 'Thornfree'	Black chokeberry 'Nero'	Jostaberry 'Josta'
Dry matter	%	11.94	20.13	14.85
Ash content	%	0.34	0.41	0.28
Total acidity	%	1.50	1.20	2.38
Total sugars	%	2.71	6.22	9.71
Vitamin C	mg/100 $g_{F.W.}$	3.11	10.49	78.34
pH		3.06	3.31	2.91
Coeff. of sweet.	(sugars/acidity)	1.8	5.2	4.1

Compared to our sample, cultivar blackberry 'Chester Thornless' from the area of Banja Luka has a slightly lower content of dry matter (10.62 %) and ash (0.32 %), and slightly lower acidity (1.40 %), but twice as much sugar (6.07 %) and seven times more vitamin C (21.36 mg/100 $g_{F.W}$), (Jazić et al., 2018). Compared to our sample, wild blackberries (*Rubus fruticosus* L.) from the area of Banja Luka are superior in all observed parameters except in sugar content (0.73 - 1.20 %), (Jazić, 2019). The acidity of fruits in our sample is in agreement with the results for the experimental orchard near Čačak, Western Serbia, where the average acidity of cultivar 'Thornfree' is 1.64 % (Milošević et al., 2012). In previously conducted research in the area of Banja Luka, the following values for 'Chester Thornless' and wild blackberries have been established, respectively: dry matter - 16.64 % and 16.72 %; total sugars - 7.03 % and 8.33 %; total acidity - 1.31 % and 0.64 % (Vasilišin & Grubačić, 2009).

Among observed taxa, blackberry leads in the content of: Mg, Fe, Mn, Cu, and Al. Black chokeberry contains the most Zn, while jostaberry is the leader in the content of: P, K, Ca, Na and Se. Cadmium and Cr were not detected, while Pb was found in black chokeberry and jostaberry in low concentrations (table 2). The content of phosphorus and potassium in blackberry `Thornfree` does not differ significantly from the content in cultivar `Chester Thornless` from the area of Banja Luka (P - 26.9 mg/100 g, K - 171.40 mg/100 g) and in wild blackberries (P - 26.8 – 27.8 mg/100 g, K - 171.9 - 172.2 mg/100 g), (Jazić et al., 2018; Jazić, 2019). This is not the case with Ca, Mg, Mn, Na and Se whose content is lower than those in two other observed taxa. On the other hand, fruits of blackberry `Thornfree` have more iron and zinc and slightly more copper. According to the authors already cited (Vasilišin & Grubačić, 2009), the content of Fe and Cu in our material is approximately the same as the content in their samples (Fe – 1.27 mg/100 g for `Chester Thornless` and 0.9 mg/100 g for the wild taxa; Cu – 0.12 mg/100 g for `Chester Thornless` and 0.37 mg/100 g for the wild taxa). According to the same source, the manganese content in our sample is significantly lower, but the zinc content is significantly higher (Mn – 0.55 mg/100 g for `Chester Thornless` and 1.18 mg/100 g for the wild taxa; Zn – 0.11 mg/100 g for `Chester Thornless` and 0.34 mg/100 g for the wild taxa).

Table 2: Elements in fresh fruits

Element	Unit	Blackberry 'Thornfree'	Black chokeberry 'Nero'	Jostaberry 'Josta'
P	mg/100 g	24.43	24.41	25.81
K		157.31	162.39	172.03
Ca		24.33	30.19	39.16
Mg		20.76	16.44	15.69
Fe		1.09	0.82	0.19
Mn		0.14	0.09	0.11
Zn		0.84	2.07	0.75
Cu		0.22	0.16	0.21
Na		2.32	1.91	2.44
Al		1.85	0.92	1.13
Se	µg/100 g	0.18	0.50	0.83
Cd		0.0	0.0	0.0
Cr		0.0	0.0	0.0
Pb		0.0	0.002	0.006

With the exception of nonflavonoids, black chokeberry stands out significantly with the content of chemical compounds from the category of phytochemicals compared to blackberry

and jostaberry, while the differences between blackberry and jostaberry are not so pronounced (table 3). It is interesting to note that the chokeberry leaf shows even higher antioxidant capacity than the fruit (Szopa *et al.*, 2017).

Table 3: Phytochemicals and antioxidant activity of fresh fruits

Parameters	Unit	Blackberry 'Thornfree'	Black chokeberry 'Nero'	Jostaberry 'Josta'
Phenols	µgGAE/mg $_{F.W.}$	5.47	74.80	5.11
Nonflavonoids	µgQcE/mg $_{F.W.}$	4.59	3.39	3,18
Flavonoids	µgQcE/mg $_{F.W.}$	0.89	71.42	1.94
Flavonols	µgQcE/mg $_{F.W.}$	0.40	0.77	0.32
Anthocyanins	µg/mL	15.35	45.92	21.04
Monomeric anthocyanins	µg/mL	13.80	40.55	19.10
ABTS	IC_{50} mg Trolox/mL	0.37	0.17	0.22
DPPH	IC_{50} mg Trolox/mL	1.45	0.97	1.89
FRAP	mmol Fe(II)/mg $_{F.W.}$	0,04	0.10	0.04

In all three tests, the highest antioxidant activity was shown by black chokeberry, which also has the highest content of phenols, flavonoids and anthocyanins. According to ABTS free radical, jostaberry shows higher antioxidant activity than blackberry, while according to DPPH free radical, blackberry has higher antioxidant capacity than jostaberry. This discrepancy is probably caused by similar concentrations of phenols and flavonols, and significantly higher amounts of vitamin C in jostaberry. According to the FRAP assay, blackberry and jostaberry have the same reduction potential of iron, which is 2.5 times lower than that of black chokeberry. Comparing the results for phytochemicals and antioxidant activity of fresh fruits with data from other sources, unfortunately they could not be carried out satisfactorily. The reason for this should be sought in the insufficient standardization of *in vitro* methods. Among other things, probes are performed in different solvents and for different periods of time (Magalhães *et al.*, 2009), samples are normalized to different standard antioxidants (Abramovič *et al.* 2018), the results are sometimes expressed in relation to fresh mass, and other times to dry mass, etc.

The results presented affirm three black-fruited bushes as valuable and healthy fruit crops, interesting not only for commercial cultivation, but also for peri-urban farming and urban gardening. In this regard, the aesthetic properties of the observed taxa are not negligible, especially blackberries and chokeberries in the flowering phenophase (fig. 1 & 2). Also, these species are imposed as useful honey plants.



Figure 1: Blackberry 'Thornfree'
(photo: S. Ljubojević)



Figure 2: Black chokeberry 'Nero'
(photo: S. Ljubojević)

Conclusions

Blackberry, black chokeberry and jostaberry have a fairly uniform elemental composition except black chokeberry, which has a significantly higher zinc content. When it comes to nutritional properties, black chokeberry has the highest content of dry matter and ash. It also has the highest pH value and the highest coefficient of sweetness. At the same time, jostaberry contains the most sugar and vitamin C, and has the highest acidity. Blackberry does not stand out in terms of any of the observed features. Regarding phytochemicals, black chokeberry dominates superiorly in the content of anthocyanins, flavonols, flavonoids, and phenols. The same is with the antioxidant capacity for all three assays: ABTS, DPPH and FRAP.

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ENVIRONMENT PROTECTION AND NATURAL RESOURCES MANAGEMENT

PREDICTION OF WATER CONDITIONS FOR MAIZE CULTIVATION ON THE CHERNOZEM SOIL UNTIL THE YEAR OF 2100

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Abstract

The aim of this paper is to estimate water conditions for maize cultivation on the chernozem soil in the area of Zemun (Autonomous Province of Vojvodina, Serbia) until the year of 2100. The study intends to evaluate future maize water requirements (ETc) and irrigation requirements. To this end, the NMMB Regional Climate Model was used. The model provided the projections of the daily minimum and maximum air temperatures, as well as rainfall for the growing seasons of 1975-2000 and 2023-2100 for the area of Zemun. The projections of these parameters were based on the RCP8.5 scenario. Based on the projected temperature, the monthly values of reference evapotranspiration (ETo) were calculated using the Hargreaves method. In addition to the plant and soil data, the obtained ETo data served as input data in the FAO CROPWAT 8.0 crop model. The examined plant was maize, while the soil was chernozem as the dominant soil type in Vojvodina. The simulations of the CROPWAT 8.0 crop model show that water requirements in the first future period 2023-2048 will be similar to the ones in the reference period (1975-2000). Irrigation requirements are even expected to be by 9% lower than in the reference period. The simulations indicate that water conditions will deteriorate in the second future period (2049-2074). The deterioration will reach its maximum in the third future period (2075-2100). Thus, the simulations show that the end of the 21st century will witness the ETc increase by 17% and the rise of irrigation requirements by as much as 73% in comparison with the reference period. It can be concluded that the simulations indicate the deterioration of the water conditions for maize cultivation on chernozem in the area of Zemun until the end of the 21st century.

Keywords: *maize, CROPWAT, climate changes, irrigation requirement*

Introduction

A noticeable increase in air temperature in the last forty years (Ruml et al., 2017) and uneven rainfall distribution in the area of Serbia show that water regime is deteriorating from the perspective of agricultural production. This is a very significant fact having in mind that the agricultural production in Serbia is mainly rainfed due to scarce irrigated land areas. The forecast of the World Meteorological Organization for Southern Europe indicates a further air temperature increase and rainfall decrease (Alcamo et al., 2007), which would reduce crop productivity (Iglesias et al., 2012). The forecast of future climate conditions in Vojvodina, the most important agricultural area of Serbia, also show the air temperature rise and rainfall decrease during summer months (Vuković et al., 2018; Lalić et al., 2011; Todorović et al., 2014), which would significantly reduce the spring crop yield (Lalić et al., 2011). Maize represents the main spring crop in Serbia, covering the largest area in comparison to other cereals and providing the highest export revenue of all agricultural products. Despite its great agronomic and economic importance, maize production has been unstable in Serbia so far (Kresović et al., 2014), with large variations in realized yields over the years. Since maize production in Serbia is mainly conducted under natural rainfed conditions, future conditions

for maize production are uncertain, having in mind the expected forecast. The aim of this paper is to estimate the water regime of the land cultivated with maize until 2100, maize water requirements and irrigation requirements, based on the climate projections regarding temperature and rainfall in the area of Zemun. The obtained results would be significant for the irrigation development planning, since stable and high maize yields in Serbia cannot be expected without the application of irrigation (Maksimović et al., 2008; Kresović et al., 2014).

Materials and methods

Climate projections for the area of Zemun were obtained using the results of the Nonhydrostatic Multi-Scale Model on the B grid (NMMB) of the regional climate model, with the horizontal resolution of 8 km (Djurdjević and Kržič, 2014). Simulations were based on the RCP8.5 (Representative Concentration Pathway) scenario, presented in the Fifth Assessment Report of the International Panel on Climate Change (Kovats et al., 2014). This scenario envisages the continuation of greenhouse gas emission, which would result in the energy imbalance of approximately 8.5 W/m^2 until the end of the 21^{st} century (Moss et al., 2010). Daily values of rainfall, minimum, maximum and mean air temperatures for the 1975-2000 and 2023-2100 periods were statistically corrected by the quantile mapping method (Piani et al., 2010; Dettinger et al., 2004) based on meteorological measurements by the Republic Hydrometeorological Service of Serbia at the station Surčin. This is a common manner for removing the systematic error (BIAS) which all numerical models contain to a certain degree. Based on the projected temperature, the monthly values of reference evapotranspiration (ETo) were calculated for these periods using the Hargreaves method (Hargreaves and Allen, 2003). Apart from the plant and soil data, the obtained ETo data served as input data in the FAO CROPWAT 8.0 crop model. The selected plant data involve the crop coefficient, length of particular growth stages, effective root depth, critical depletion fraction, yield reduction coefficient and potential plant height. The plant parameters are in accordance with FAO56 (Allen et al., 1998). The assigned maize sowing date was April 10, while September 6 was the harvest date. Chernozem soil of the Zemun loess terrace was selected, with the 170 mm/m soil available water capacity. The calculations were conducted assuming that soil moisture on the sowing date was on the field capacity level. Effective rainfall was calculated using the fixed percentage method (90% of the total rainfall). The study processed the results of the CROPWAT simulations on the level of the growing season (April 10-September 6). The following data were presented: potential maize evapotranspiration (ETc), actual maize evapotranspiration (ETa), effective rainfall, maize irrigation water requirement. The future period was divided into three same-length periods: 2023-2048, 2049-2074 and 2075-2100. The analysis was performed comparing the mean value of a certain parameter of each future period and the mean value of the selected reference period (1975-2000). In order to eliminate the impact of the biases caused by the climate model, the simulated future values were compared with the corresponding ones in the simulated reference period rather than with the gauged values (Shen et al., 2018). The changes were presented as relative, expressed in %. In the graphs representing time series of normalised anomalies relative to the 1975–2000 mean, the curves of ten-year moving averages were inserted in order to detect change tendencies.

Results and discussion

According to the climate model projections, the growing season temperatures during the reference period (1975-2000) are in the range from 12.4 to 24°C. The mean daily temperature

of the growing season (Tmean) is 18.3°C. The rise of Tmean is expected in all three future periods (Tab.1). Tmean will increase period after period, with the projected significant warming at the end of the 21st century. A study by Vuković et al. (2018) also indicates the accelerated air temperature increase by the end of the 21st century.

Tab.1. Projected average values of mean (Tmean), maximum (Tmax) and minimum temperature (Tmin) of the maize growing season and their deviation in °C from the reference period (1975-2000)

Period	Tmax (°C)	Tmin (°C)	Tmean (°C)	Tmax	Tmin	Tmean
				Deviation from the reference period (°C)		
1975-2000 ref	24.0	12.4	18.3			
2023-2048	25.0	14.2	19.8	1.0	1.7	1.5
2049-2074	26.6	15.9	21.7	2.5	3.4	3.4
2075-2100	30.7	20.0	27.0	6.7	7.5	8.8

Contrary to Tmean which shows the growing tendency in all future periods, the projected effective rainfall of the growing season (P_{eff}) will rise in the first period in comparison to the reference period. In the other two future periods, effective rainfall is anticipated to drop. The end of the 21st century is expected to witness a 34.1% reduction in comparison to the P_{eff} in the reference period (Tab.2).

Tab. 2. Projected average effective rainfall (Peff), potential (ETc) and actual (ETa) maize evapotranspiration and irrigation requirements (Irr.req.) and their deviation in % from the reference period (1975-2000)

Period	P_{eff} (mm)	ETc (mm)	ETa (mm)	Irr.req. (mm)	P_{eff}	ETc	ETa	Irr.req.
					Deviation from the reference period (%)			
1975-2000	258	511	353	253				
2023-2048	278	516	354	230	7.8	1	0.3	-9.1
2049-2074	221	536	299	315	-14.3	4.9	-15.3	24.5
2075-2100	170	606	277	437	-34.1	18.6	-21.5	72.7

Rain distribution shows no regularity in the entire future period. The time series of normalised anomalies of the effective rainfall relative to the 1975–2000 mean shows an increasingly negative deviation (reduction) over time (Fig.1). The greatest predicted positive deviation will be approximately 100%, while the negative deviation will amount to 86% at the end of the century. The ten-year moving averages curve shows the falling tendency.

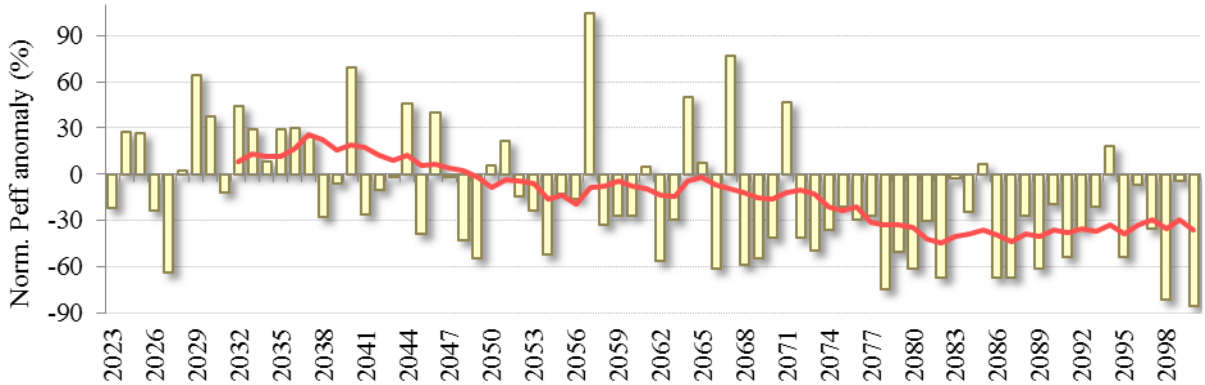


Fig. 1. Time series of normalised anomalies of the projected effective rainfall (P_{eff}) in the area of Zemun during the maize growing season 2023-2100 relative to the 1975–2000 mean. The curve shows the moving average values for a 10-year period assigned to the last year of the period.

Table 2 presents the projected average maize water requirements (ETc) in the three future periods and in the reference period in the Zemun area. The deviation from the reference period is insignificant (1%) in the 2023-2048 period. In the second and third periods the ETc deviation is greater and amounts to 4.9% and 18.6%, respectively. The time series of normalised anomalies of maize water requirements relative to the 1975–2000 mean shows the prevalence of positive deviation (increase) in the 2049-2074 period, which continues in the third period. At the end of the 21st century, the predicted positive deviations will rise and reach their maximum in 2098 (64%). The curve of ten-year moving averages mainly shows the rising trend (Fig.2). The study of Todorovic et al. (2014) shows that in the 2035-2065 period the former Yugoslavia countries might experience a decrease of the maize crop evapotranspiration by 1%-5% in comparison to 1991-2010. The differences between our results and the results of these studies are the consequence of applying different climate models and different gas emission scenarios.

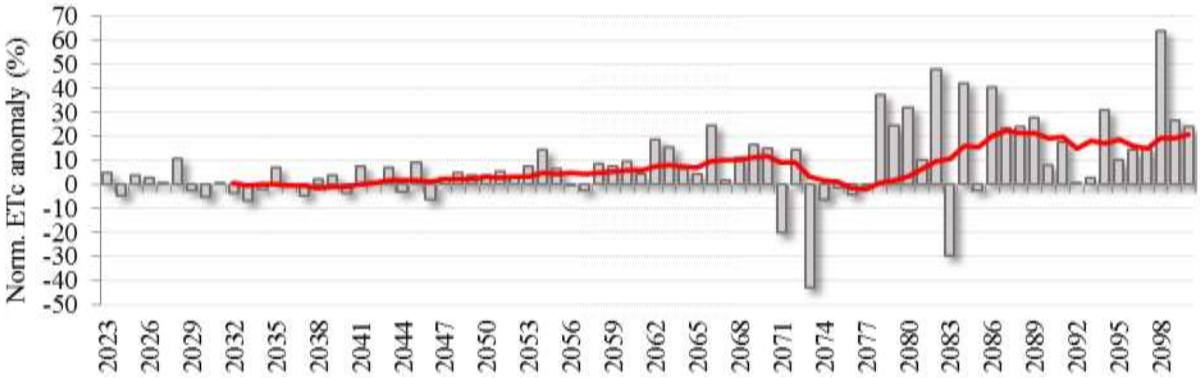


Fig. 2. Time series of normalised anomalies of the projected potential maize evapotranspiration (ETc) in the area of Zemun during 2023-2100 relative to the 1975–2000 mean. The curve shows moving average values for a 10-year period assigned to the last year of the period.

In contrast to the rising ETc tendency, the projected actual maize evapotranspiration (ETa) has the falling trend (Fig.3). In the 2023-2048 period, the time series of normalised anomalies of ETa relative to the 1975–2000 mean has a slightly positive deviation. In the other two periods, the normalized ETa anomaly is dominantly negative. The projected average ETa of the growing season relative to the 1975–2000 mean has the largest deviation of -21.5% in the 2075-2100 period (Tab.2).

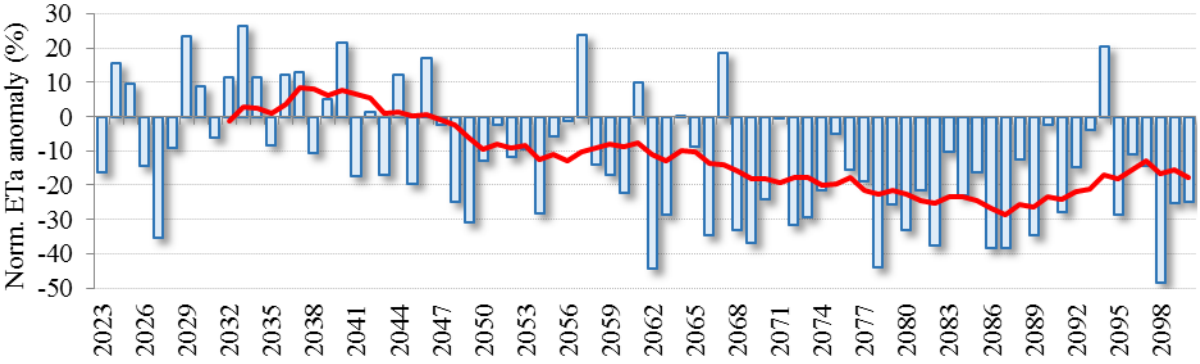


Fig. 3. Time series of normalised anomalies of the projected actual maize evapotranspiration (ETa) in the area of Zemun in the maize growing season during the research period (2023-

2100) relative to the 1975–2000 mean. The curve shows moving average values for a 10-year period assigned to the last year of the period.

The projected average values of maize irrigation requirements for all three periods are presented in Table 2. In the 2023-2048 period, the mean irrigation requirements relative to the 1975–2000 mean are predicted to drop by -9.1%. This is the consequence of the increase in the projected P_{eff} (7.8%) and relatively small ETc deviations (1%). In the other two periods the projected irrigation requirements will rise significantly relative to the 1975–2000 mean and will have the value of 72.7% at the end of the century. The 2075-2100 period is characterized by extremely high positive deviations in certain years, so the deviations greater than 80% are registered during 10 years (Fig.4). Todorović et al., (2014) show that in the area of Serbia the maize net irrigation requirements will decrease by 2% in the 2035-2065 period in comparison to 1991-2000. Increase in net irrigation for the area of Vojvodina was also determined in the research by Tovjanin et al., (2019). Simulating the maize production under climate change conditions, they obtained a very significant 183 mm increase in the net irrigation for the 2071-2100 period compared to 1961–1990. The curve of the ten-year moving averages mostly shows a rising tendency (Fig.4).



Fig. 4. Normalised deviation of the projected maize irrigation requirements in the area of Zemun in 2023-2100 relative to the 1975–2000 mean. The curve shows moving average values for a 10 year-period assigned to the last year of the period.

The projections obtained in the CROPWAT model depend on the applied climate models and climate scenarios. Thus, the application of the ECHAM5 climate model with the SRES-A2 scenario for greenhouse gas emissions for the 2030 in the area of Vojvodina (Lalić et al., 2013) predicts an approximately 20% lower P_{eff} than in the period 1971-2000. In our research, P_{eff} projections (obtained using the NMMB climate model with the RCP8.5 scenario) are by approximately 8% higher in the first future period than in the reference period (Tab.2). Different rainfall projections cause different CROPWAT simulations of ETc and irrigation requirements. Maize ETc on chernozem (Lalić et al., 2013) has an approximately 60% increase, while irrigation requirements rises by more than 150% compared to the reference period. Our study shows different predictions for the first future period. ETc will be higher by only 1%, while Irr.req. will decrease by 9% compared to the reference period. A larger number of models and different scenarios would ensure more precise future estimation of conditions.

Conclusion

The CROPWAT simulations of the parameters related to maize production under climate changes in the area of Zemun show that the conditions of chernozem water regime will

deteriorate until the end of the 21st century. In the first future period (2023-2048), the conditions are expected to be similar to the conditions from the reference period (1975-2000), while water regime will deteriorate until the end of the 21st century. Maize water requirements (ETc) will increase, while actual evapotranspiration (ETa) will have a falling tendency due to the reduction of available water. This situation will result in the growing need for maize irrigation, which will be more than 70% higher at the end of the 21st century than in the reference period. The presented results are burdened by uncertainty typical of all climate models. The application of ensemble climate models would reduce uncertainty, which could represent a subject of future research.

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CONTENT OF POTASSIUM IN SOIL ON THE TERRITORY OF NIŠ TOWN MUNICIPALITIES IN SOUTHERN SERBIA

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Abstract

Potassium is considered to be one of the most significant biogenic elements which is essential for the plant growth and which has positive effects on plant's disease resistance. It is also beneficial for the root growth and improves drought resistance, maintains turgor, reduces the water loss and the respiration by preventing energy losses, enhances the translocation of sugars and starch. It belongs to the group of alkali metals and its natural reserves are large. However, the readily available forms of this element are commonly found in small quantities. Soluble potassium, i.e. soil solution potassium is the form most available to plants. It is present in the soluble salts of the soil solution as well as in the ionian solution which is balanced with the salts absorbed by the colloidal complex. This paper aims to examine the soil content of the readily available forms of potassium on the territory of Niš town municipalities (southern Serbia) in 2015. The total number of the collected samples was 307. The collected samples were examined by the AL-method in the laboratory of the Agricultural and Advisory Services in Niš. The obtained results were processed by the computer program IBM SPSS Statistic – 20 (trial version). The analysis proved that the soil in the examined region is rather rich in the readily available potassium. Almost 63% of the samples were classified as those containing high levels of potassium while only 2% of the total samples were classified as those with low levels of potassium.

Key words: *potassium, analysis, soil, Niš town municipalities*

Introduction

Minerals play various roles in the plant growth. Around 70 elements have been discovered to be present in plants, some of which are essential while the others are regarded as useful elements. For an element to be considered essential to the plant, certain conditions are to be fulfilled: its deficiency prevents a further growth of the plant; it causes the plant chronic growth and development; its deficiency symptoms are more or less typical (Kastori i Maksimović, 2008). Potassium is one of these elements. It belongs to the group of alkali metals and its natural reserves are rather large. It is present in plants and soil as the monovalent cation (K^+) with reduction characteristics. It is not an integral part of the organic matter but is predominantly linked to the proteins. Potassium is vital to many plant processes, which makes it a crucial biogenic element (Jelić, 2012). Potassium is beneficial for the root growth and improves drought resistance; it maintains turgor; it reduces the water loss and the respiration by preventing energy losses; it enhances the translocation of sugars and starch; it produces grains rich in starch, increases protein content of plants, builds cellulose and reduces lodging while simultaneously helping in the disease resistance (Radulov et al, 2012). The supply of potassium in soil is large, ranging from 0.2% to 3.0%, but only a small percentage is available to plants. This potassium originates from various primary minerals which

decompose during the process of pedogenesis and are transformed into secondary minerals or clay minerals. Therefore, the clay quantity is regarded as an indicator of the potassium supply in soil (Gudžić, 2015). Soluble potassium, i.e. soil solution potassium is the form most available to plants. It is present in the soluble salts of the soil solution as well as in the ionian solution which is balanced with the salts absorbed by the colloidal complex (Radulov et al, 2014). The percentage of the potassium present in the water soil solution is only 1% out of the total quantity of the exchangeable potassium. This leads to the conclusion that the quantities of the soluble potassium are rather small and insufficient for the plant use (Kastori et al, 2013). The soil solution potassium provides only 5% of the total plant demand (McLean i Watson, 1985) and represents 0.1% out of 0.2% of the presently available potassium, which is complemented by the exchangeable potassium (readily available, slowly and quickly available) or the non-exchangeable potassium, i.e. slowly available potassium. The exchangeable potassium rate is from 1% to 2%, while the slowly exchangeable potassium rate is from 1% to 10% out of the total potassium supply and represents the main source of potassium for plants (Römheld i Kirkby, 2010). The amount of potassium that the plant removes from the soil during the growing season depends on the plant species. So for plum which is the most widespread cultivated fruit species in Serbia (Grčak et al., 2017), an average dose of 60 to 70 kg / ha K_2O is recommended (Ubavić, 1996).
The aim of this research was to examine the soil condition on this territory in 2015 regarding the available potassium supply in soil that is used for agriculture. This would help farmers to better plan their plant production and fertilization in this area.

Material and methods

The paper studies and analyzes 307 soil samples taken on the territory of Nis municipalities. Soil samples were taken from a depth of 0-30 cm in field crops and vegetable cultures, or 0-60cm in orchards. The samples were taken from agricultural soils, and were collected on the territory of the following municipalities : the greatest number of samples were collected on the territory of Crveni Krst (84) and then the city of Nis (65), Palilula (55), Pantelej (54), Medijana (39) i Niška Banja (10), Table 1. The greatest number of samples were collected in the municipality Crveni Krst, 84, which represented 27,4% of the total number of samples (Table 1). The smallest number of samples were obtained from the municipality Niška Banja – 10 samples, i.e. 3,3% of the total number of examined samples. The total area of these municipalities is 597 km^2.
he data were statistically processed by the computer program IBM SPSS Statistic, version 20 - trial version.

Table 1. The number of samples and their percentage as regards the municipalities in which they were collected

Municipalities	Number of samples(n)	Percentage (%)
Crvani Krst	84	27.4
Grad Niš	65	21.2
Palilula	55	17.9
Pantelej	54	17.6
Medijana	39	12.7
Niška Banja	10	3.3
Total	**307**	**100**

The soil samples were analyzed by the Agricultural and Advisory Services in Niš in 2015. Prior to the chemical analysis, the samples had to be well prepared for it. The soil samples

were thus first placed in separate vessels and left to dry. The elimination of impurities, soil grinding and griddle followed. Having confirmed that the soil was free from any impurities and homogeneous, it was returned to the vessels and prepared for the chemical analysis.
There are a lot of methods used for determining the potassium supply in soil. The AL Egner-Riehm method is considered more convenient in comparison to the other ones (Manojlović et al, 1969), since the readily available phosphorus and potassium are determined from the same extract. This method has been officially recognized by a lot of countries. Therefore, this method was used in this research in order to determine the readily available potassium supply in soil, i.e. the quantity of the soil solution potassium and of the adsorptive complex potassium. The method is based upon the element extraction from the soil samples using the extraction means. The AL solution is actually the solution of 0,1 N ammonium lactate and 0,4 N glacial acetic acid (Predić, 2011). Extraction was performed by AL solution and flame photometric method was used to determine concnetration of potassium from this exctract.

Results and discussion

The obtained concentrations of available potassium were compared to the values presented in Table 2 to determine the soil supply with the readily available potassium forms and to classify the soils that our samples were taken from. Table 3 contains all 307 samples whose values were divided into three classes, according to the criteria shown in Table 2.

Table 2. Soil classification as regards the readily available potassium supply showcased by Jelić (2012)

Soil classification	Values mg K_2O $100g^{-1}$ of soil
Well supplied soil	> 20
Medium supplied soil	10 – 20
Poorly supplied soil	< 10

Table 3. Soil classification as regards the quantity of the readily available potassium obtained in all examined municipalities of Niš

Soil classification	Number of samples (n)	Percentage (%)
Well supplied soil	193	62.9
Medium supplied soil	109	35.5
Poorly supplied soil	5	1.6
Total	307	100

The analysis of all samples gave positive results presented in Table 3 . As many as 63% of samples (193 samples in total) belong to the class of the well-supplied soil. Around 35% of samples (109 samples) were classified as moderately-supplied soil by potassium and only 2% of all examined samples (5 samples) were classified as coming from the insufficiently-supplied types of soil as far as potassium was concerned.

Conclusion

When deciding which plant to grow, it is crucial to know the chemical composition of the soil, in order to avoid excessive fertilization and because different plants have different needs regarding amount of mineral elements for their growth. In conclusion, the majority of agricultural soil on the territory of the town of Nis and its municipalities is well-supplied with available potassium (62.9%) and there is no need for an additional potassium supply. This

shows that agricultural soil in this area is well nourished. Only 1.6% of samples were found to be classified as poorly supplied soil. It is certainly preferable that all agricultural households perform the soil analysis needed for the purposes of applying an efficient fertilizing method in accordance with the crops they would like to grow.

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THE EFFECT OF ZEOLITE ON A LIGHT SOIL'S MOISTURE CHARACTERISTIC CURVE

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Abstract

Water scarcity is a common phenomenon in the European Union. The Mediterranean countries, in particular, are facing high water stress, and climate change effects further exacerbate the problem. Agriculture uses over 70% of globally allocated freshwaters for irrigation. Potential irrigation water savings is, therefore, of paramount importance. Zeolites are known for their water holding capacity. Their application to agricultural soil is expected to raise soil moisture, thus leading to irrigation water savings. The aim of the present study was to evaluate the effect of a natural zeolite (85% clinoptilolite) on the water holding capacity of a loamy sand soil, via the estimation of the soil moisture characteristic curve (SMCC). A SMCC is an important hydraulic property, playing a key role in water management. The SMCCs were estimated in the laboratory under controlled conditions, applying known pressures to soil cores, using a pressure membrane extractor. The soil cores were produced from disturbed soil samples. Two application rates of zeolite were used (0.1 and 0.5% w/w) and non-amended soil was used as control. The results showed that the addition of zeolite increased soil moisture content at all applied pressures up to 3 bars. The application of zeolite to a light soil, even at these relatively low rates of 0.1 and 0.5% w/w, increased the available water holding capacity by 26% and 39%, respectively, thus indicating significant irrigation water savings.

Keywords: *Field capacity, Permanent Wilting Point, Available moisture, Loamy sand soil, Clinoptilolite.*

Introduction

Fresh water is becoming scarce not only in arid and drought prone areas, but also in areas with abundant precipitation. Water scarcity is a widespread phenomenon in the European Union. Already by 2007, water scarcity has affected a minimum of 11% of Europe's population and 17% of its territory (EC, 2019). Agriculture uses over 70% of globally allocated freshwaters for irrigation. In parts of southern Europe, agriculture has been estimated to account for around 80% of total water abstraction (EEA, 2009). The sustainable use of water is a priority for agriculture in water scarce regions. Water management in these regions involve, among others, to reduce water demand or make water use more efficient. Higher efficiency means that less water should be abstracted from surface or groundwater bodies to produce a certain yield, thus producing water savings (Pereira *et al.*, 2002).

Natural zeolites are crystalline hydrated aluminosilicates. The name "zeolite" means boiling stone and derives from the Greek words "ζέω" (zeo: boil) and "λίθος" (lithos: stone). The structure of the zeolites comprises a three-dimensional network mainly consisting of aluminosilicates with SiO_4 and AlO_4 tetrahedra connected with each other by sharing all oxygen atoms (Breck, 1974). Due to the high porosity of their crystalline structure, zeolites may hold water more than half of their weight. Water molecules in the pores could easily be evaporated or reabsorbed without damage to the structure (Sangeetha and Baskar, 2016).

Zeolites' properties that are related to their structure include among others, high potency of hydration, cation exchange capacity and high internal surface area available for adsorption (Eroglu *et al.*, 2017). Clinoptilolite is one of the most common natural zeolites, widely distributed and used throughout the world (Polat *et al.*, 2004). Natural zeolites can positively affect several soil properties, including soil moisture (Eroglu *et al.*, 2017). Their porous structure assures a permanent water reservoir, resulting in prolonged moisture during dry periods. Zeolites also contribute towards prompt rewetting and improve the horizontal spread of irrigation water within the root zone, thus leading to irrigation water shavings (Polat *et al.*, 2004). Zeolites can improve, among others, the water retention of sandy soils (Ghazavi, 2015; Eroglu *et al.*, 2017). Soil hydraulic properties are essential in irrigation and drainage studies. A soil moisture characteristic curve (SMCC) is an important hydraulic property that describes the amount of water retained in a soil (expressed as volumetric water content) under equilibrium at a given matric potential. Modeling water distribution and flow in partially saturated soils requires knowledge of the SMCC, hence playing a critical role in water management (Tuller and Or, 2005). Available water-holding capacity (AW) of a soil is also an important hydraulic property, as it quantifies the amount of water available for plants that the soil can hold. The AW is defined as the amount of water held by the soil between field capacity (FC) and permanent wilting point (PWP) and can be estimated from the SMCC (Blaschek *et al.*, 2019). The objective of the present study was to evaluate the effect of a natural zeolite (85% clinoptilolite) on the water holding capacity of a loamy sand soil, via the estimation of the soil moisture characteristic curve.

Materials and methods

The soil used in this study was loamy sand (83% sand, 6% clay and 11% silt). Soil organic matter content was 0.27%. Soil samples were air-dried and sieved to less than 2 mm.
The zeolite used was Bulgarian, commercially available, granular, natural zeolite with minimum clinoptilolite content of 85%. The particle size of the zeolite was originally within the range of 2.5-5.0 mm. We used granular, rather than powder zeolite (0-0.063 mm), as this is the one most often used for applications to agricultural soils. However, the granular zeolite was ground and sieved to less than 2 mm (Figure 1), in order to achieve better uniformity within the soil-zeolite mixtures.



Figure 1. Granular zeolite with particle size less than 2 mm.

To produce the soil-zeolite mixtures, two application rates of zeolite were used (0.1 and 0.5% w/w). Non-amended soil was used as control. All three treatments were replicated three times. Non-amended soil (LS) and soil-zeolite mixtures (LS+0.1%Z and LS+0.5%Z) were placed in metallic retaining rings. The dimensions of the rings were as follows: diameter of 5.4 cm and height of 3.0 cm.

The SMCCs were estimated in the laboratory under controlled conditions, applying known pressures to soil cores (non-amended soil and soil-zeolite mixtures in the retaining rings), using a pressure plate extractor. The pressure plate extractor used (Figure 2) comprises of two ceramic pressure plate cells, one for lower pressures up to 5 bars and the other for pressures up to 15 bar (Soilmoisture Equipment Corp.). Each pressure cell encloses a water-saturated porous plate, which allows water but not air to flow through its pores. The porous plate is at atmospheric pressure at the bottom, whereas the top surface is at the applied pressure of the cell. During a run at any set air pressure in the extractor, soil moisture will flow from around each of the soil particles and out through the ceramic plate. This flow continues until the effective curvature of the water films throughout the soil are the same as at the pores in the plate. When this occurs, equilibrium is reached and the flow of moisture ceases. At equilibrium, there is an exact but opposite relationship between the air pressure in the extractor and the soil suction (moisture content) in the samples (Young, 1983; Tuller and Or, 2005).



Figure 2. Pressure membrane extractor used to estimate the SMCCs.

Soil cores were placed in contact with the porous plate and allowed to saturate by immersion in deionized water for 48 hours. Samples were weighed at saturation and placed back to the porous plate. The porous plate with saturated soil samples was then placed in the chamber. A set of known pressures (0.1, 0.2, 0.3, 0.5, 1, 2, 3, 5, 10 and 15 bar) was applied to force water out of the soil through the plate. The equilibrium between soil matric potential and the applied air pressure was determined by weighing the cores, till constant weight was achieved. Following equilibrium at the final pressure of 15 bar, soil cores were oven-dried at 104 °C for 48 hours to determine the mass water content gravimetrically. These values were then converted to volume water contents using the sample bulk densities.

The data set produced from the procedure described above was used to develop the SMCC for

each treatment. The curves were then used to quantify the availability of soil moisture for plant growth. Firstly, field capacity (FC) and permanent wilting point (PWP) were estimated at 0.33 and 15 bar, respectively. Finally, the available water holding capacity (AW) of the soil was then calculated as the difference between FC and PWP.

Results and discussion

The SMCCs, which were developed for the three treatments, within the suction range of 0.1 to 15 bar, are presented in Figure 3. The values shown in Figure 3 are average of three replicates. The SMCCs follow the slope of a typical SMCC for sandy soils.
The results showed that the addition of zeolite, even at the low rate of 0.1% w/w, increased soil moisture content at all applied pressures up to 3 bars. From the pressure of 5 bar and up to the pressure of 15 bar, the soil moisture content was practically constant and rather low (3% v/v) for all treatments.

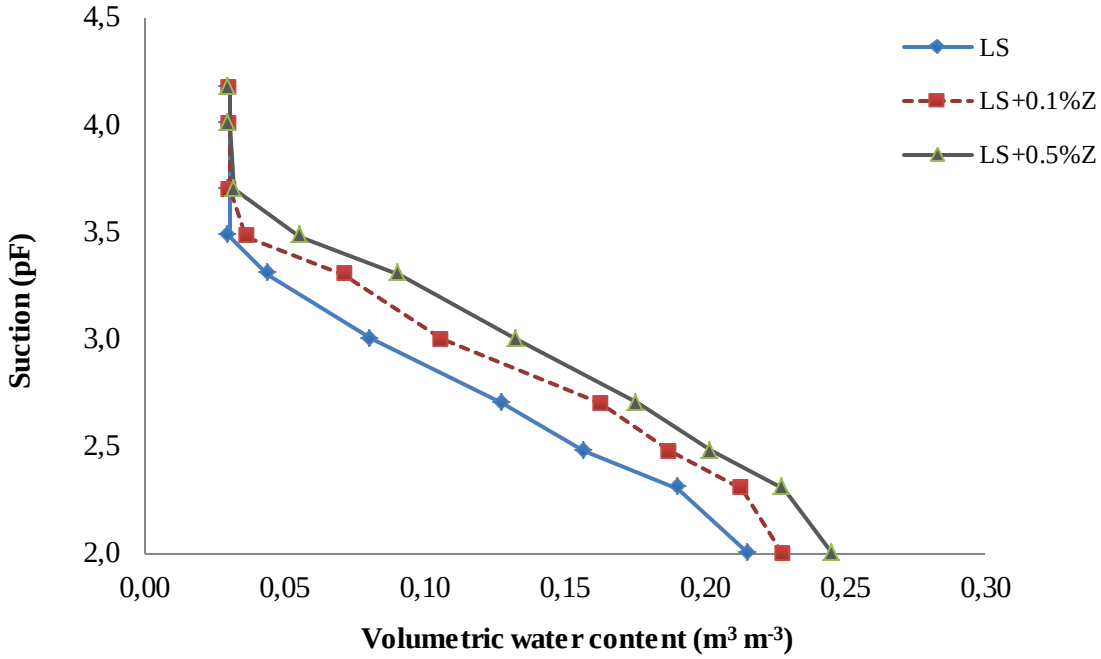


Figure 3. The soil moisture characteristic curves (SMCCs) of the three treatments: non-amended soil (LS), soil and zeolite mixture 0.1% w/w (LS+0.1%Z) and soil and zeolite mixture 0.5% w/w (LS+0.5%Z).

Although the volumetric soil moisture content at saturation was about 39% for all treatments (Figure 4), at the suction of 0.1 bar, the volumetric soil moisture content was reduced by 41% on average. This finding can be explained by the fact that the large pores of the loamy sandy soil empty rapidly at slightly negative matric pressure resulting in low moisture retention (Nimmo, 2004).

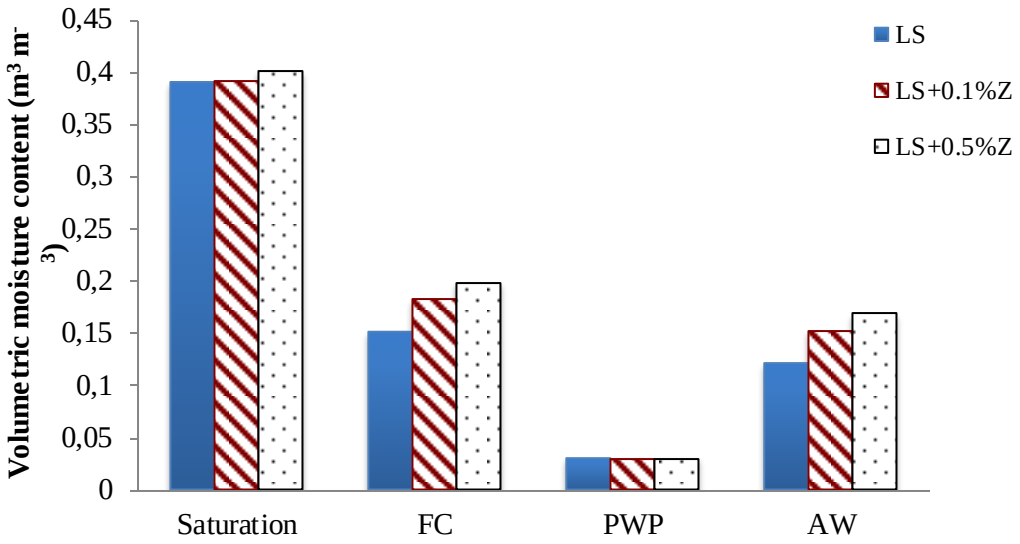


Figure 4. Volumetric soil moisture content at saturation, field capacity (FC), permanent wilting point (PWP) and available water (AW) for the three treatments: non-amended soil (LS), soil and zeolite mixture 0.1% w/w (LS+0.1%Z) and soil and zeolite mixture 0.5% w/w (LS+0.5%Z).

As it can be seen in Figure 4, the application of zeolite at the rate of 0.1% w/w to the loamy sand soil increased the available water holding capacity by 26%, whereas at the rate of 0.5% w/w by 39%. These findings indicate significant irrigation water savings.

Conclusion

This study evaluated the effect of a natural zeolite (85% clinoptilolite) on the water holding capacity of a loamy sand soil, via the estimation of the soil moisture characteristic curve (SMCC). Two application rates of zeolite were used (0.1 and 0.5% w/w) and non-amended soil was used as control. The results showed that the application of zeolite increased soil moisture content at all applied pressures up to 3 bars. The addition of zeolite at the rates of 0.1 and 0.5% w/w increased the available water holding capacity by 26% and 39%, respectively, thus indicating significant irrigation water savings.

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IS THE EXPLOITABLE BIOMASS (*B*) VULNERABLE TO CHANGES OF NATURAL MORTALITY (*M*) BY AGE? CASE OF SARDINA PILCHARDUS (WALBAUM, 1792)

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Abstract

For this study, 3571 individuals of all sexes, with sizes ranging from 7.25 to 17.75 cm, were studied. Monthly sampling was conducted from December 2018 to March 2020 from landings of seiners operating in the central region of Algeria between Tenes and Bejaia. To feature a potential effect of changes in characteristic mortality (*M*) on the biomass level of *Sardina pilchardus*, it was important to estimate its value for the entire population at first and then for each age class. The VPA was then run with a constant estimation of *M* [0.6 yr^{-1}] and a variable one [from age 1 to 4 = 1.466; 0.818; 0.680; and 0.591 yr^{-1}]. The outcomes appeared for both constant and variable *M* a condition of "not overfishing and not overfished", with, for constant *M* a biomass estimation of 5636 tons and 6453 tons for variable *M*. The addition in biomass level estimation was around 13%, when the estimation of the yield per year stayed fundamentally the equivalent [around 2912 tons]. Finally, we recommend incorporating changes of natural mortality (*M*) in biomass estimation studies to get closer to reality. Indeed, at younger age small pelagic fishes are the most vulnerable classes to mortality caused by predation or pollution induced stress. This fact must be taken into serious consideration for a better monitoring of exploitable stocks, without ignoring the degree of uncertainty of estimates.

Keywords: *Sardina pilchardus, Algeria, M, Biomass.*

Introduction

Globally, fisheries and aquaculture make a substantial contribution to the food security of millions of people. Excluding aquatic plants, total global production in the sector peaked at 171 million tons in 2016; 53% of this total comes from capture fisheries (FAO, 2018).
In response to such a demand, fisheries science must try to estimate, as faithfully as possible to reality, the biomass currently available for exploitation in order to help decision-makers to adopt the right fishing strategies to meet this demand without compromising the renewal of fish stocks in the long term. A monospecific population has a certain number of biological characteristics which are specific to it, such as age, different growth parameters, as well as its frequency of mortality, which is either caused by fisheries (*F*), or natural (*M*). In reality, the values of the natural mortality (*M)* are not constant but do vary according to age. The main objective of the present work was to study the variations in the exploitable biomass estimates of sardines (*Sardina pilchardus*, Walbaum, 1792) in the central region of the Algerian coast according to variations in *M* by age. And therefore, to try to acquire scientific knowledge on how these variations can impact population dynamics and the evaluation of exploited marine stocks.

Materiel and Methods

To asses the state of the sardine stock in the central region of the Algerian coast, 3571 fishes with sizes ranging from 7.25 to 17.75 cm all sexes combined, were sampled from December 2018 to March 2020 from the landings of purse seiners operating in the central region of Algeria between Tenes and Bejaia. The determination of the age-length key of the sample was carried out using the FISAT II 1.2.2 by the Bhattacharya's method whose protocol was slightly modified by Gayanilo et al. (2005). The age-length key made it possible to determine the parameters of the linear growth of the von Bertalanffy model (1938) using New VONBIT for Excel software (Stamatopoulos, 2012). The model can be written as the following:

$$\boldsymbol{L_t = L_\infty \left(1 - e^{-K(t-t_0)}\right)}$$

where: ($\boldsymbol{L_\infty}$) represents the asymptotic length, ($\boldsymbol{K}$) the growth coefficient and ($\boldsymbol{t_0}$) the age at which $L_t = 0$.

To determine the length-weight relation, 20 pairs of values total length (L_t) – total weight (W_t) were considered, using the following equation: $\boldsymbol{W = a\, L^{\,b}}$. According to Bouaziz et al. (1998) and Froese et al. (2011), to confirm or deny the allometry, the application of the *t*-test based on the comparison between the calculated slope *(b)* and the theoretical slope (that equals 3 in this case) is necessary.

For the total mortality (*Z*), it was estimated by FISAT II using the method of Pauly (1990) based on the length-converted catch curve.

The value of natural mortality (*M*) was estimated by the method of Djabali et al. (1994). The choice fell on this method due to the fact that the equation was adapted from Pauly's (1980) to Mediterranean stocks.

$$\boldsymbol{\log_{10} M = 0.0278 - 0.1172 \times \log_{10} L_\infty + 0.5092 \times \log_{10} K}$$

The value of mortality *M* was calculated first for the entire population (constant *M* value) and subsequently for each age class. To do this, it was first necessary to estimate the values of *L*∞ and *K* for each cohort.

- Asymptotic length (*L*∞*)*:

The asymptotic length of each age class was calculated using the method of Taylor (1962):

$$\boldsymbol{L_\infty = \frac{L_{max}}{0.95}}$$

- Growth coefficient (*K*):

For the calculation of the growth speed by age, the equation used was deduced from the von Bertalanffy (1938) model: $\boldsymbol{L_t = L_\infty \left(1 - e^{-K(t-t_0)}\right)}$

$$\boldsymbol{K = \frac{-Ln\left(\frac{L_\infty - L_t}{L_\infty}\right)}{(t - t_0)}}$$

The value of fishing mortality *F* is then deduced from the equation of Z: $\boldsymbol{Z = F + M}$.

Another data necessary for the estimation of the spawning biomass is the percentage of mature individuals by size class (Table 1). The data was taken from Bouaziz et al. (2014) histological study on *S. pilchardus*.

Finally, in order to assess the state of the fish stock and palpate the effect of fishing on it, with both constant and variable values of natural mortality (*M*), we used the software recommended by FAO, namely VIT4win version 1.3 (Lleonart & Salat, 2011). This software is designed for Mediterranean fisheries and has the advantage of working with pseudo-cohorts, that is to say, it only requires knowledge of the catches in one year instead of a long historical series of ten years.

Results and discussion

- Calculation of biological parameters and mortality

The Bhattacharya method splits the sample of *Sardina pilchardus* into four cohorts with lengths oscillating around 9.87, 12.41, 15.51, and 17.62 cm (Table 1).

The results of the parameters of linear growth, mortalities (*Z*, *M*, and *F*) and the percentage of mature individuals, both sexes, are shown in Table 1 and Figures 1 and 2.

Table 2. Data needed for the calculation of the exploitation according to the length of *S. pilchardus*, from the central region of the Algerian coast

Age (yr)	ML (cm)	n	CM ± SD (cm)	L_∞ (cm)	K (yr^{-1})	M (yr^{-1})	MI	Parameters
1	7.5	12	9.87 ± 1.05	10.26	3.12	1.47	0	von Bertalanffy's equation (New VONBIT for Excel) $L_t = 18.498\left(1 - e^{-0.64(t+0.021)}\right)$ where: L_∞ = 18.498 cm, K = 0.64 yr^{-1}, t_0 = - 0.021 yr
	8	21					0	
	8.5	35					0	
	9	57					0	
	9.5	78					0	
2	10	81	12.41 ± 0.87	13.95	1.09	0.82	0.05	Allometry equation $W_T = 0.0035\ L_T^{\ 3.2651}$ where: a = 0.0035, b = 3.2651
	10.5	102					0.12	
	11	220					0.27	
	11.5	270					0.38	
	12	260					0.49	
	12.5	280					0.57	
	13	302					0.63	Mortality estimation (FISAT II 1.2.2)
	13.5	174					0.69	
3	14	189	15.51 ± 1.17	17.11	0.79	0.68	0.86	Z = 2.34 yr^{-1}
	14.5	197					0.91	M = 0.60 yr^{-1} (Djabali et al. 1994)
	15	217					0.98	F = 1.74 yr^{-1}
	15.5	309					1	
	16	326					1	
	16.5	206					1	
4	17	147	17.62 ± 0.77	19.21	0.62	0.59	1	
	17.5	88					1	

ML = mid-length; CM = computed mean; SD= standard deviation; MI = mature individuals; *n* = number of fish

Regarding the study of the length–weight relation, the *t*-test based on the comparison of two slopes provided a value of 6.06 (> to 2.083 for α = 5%). This result allows us to conclude that sardine of both sexes, captured in the study area, was characterized by a major allometry.

The high value of *K* (0.64 yr^{-1}) corresponds to the theory of Sparre and Venema (1996), which concluded that small pelagic fish, such as sardines, are characterized by rapid growth so implying a high value of *K* and a low longevity. A decrease in its value by age is also observed (Fig. 1). It also indicates a significant growth coefficient for young sardines compared to older individuals.

Natural mortality (*M*) also decreases with age (Fig. 2), and young sardines are the most prone to it. This variation could be explained by their vulnerability to variations in environmental conditions such as temperature and salinity. In addition, due to their small size, young individuals become an easy prey for many predators (Garrido, 2016 ; Quattrocchi & Maynou, 2017).

Bouaziz (2006) noted that for small pelagics, the values of fishing mortality (*F*) that maximize the yield per recruit are generally very high. This agrees perfectly with the value of *Z* chosen, which was 2.34 yr^{-1}.

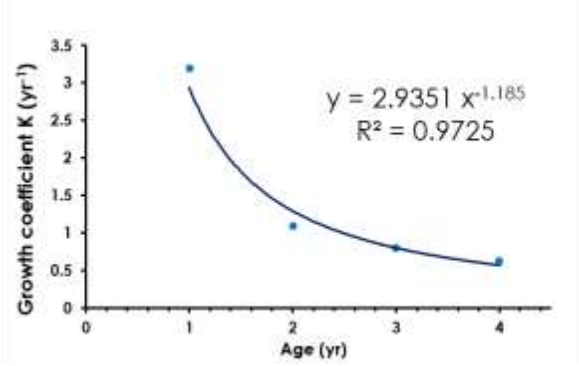


Figure 1. Evolution of growth coefficient (*K*) of *Sardina pilchardus* from the central region of the Algerian coast, according to age.

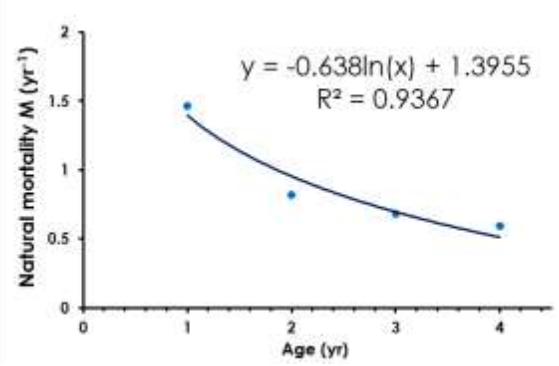


Figure 2. Evolution of natural mortality (*M*) of *Sardina pilchardus* from the central region of the Algerian coast, according to age.

- Main results of the VPA

*Biomass and yield per recruit***.** The VPA made it possible to determine the yield per recruit as well as the average total biomass per recruit. Figure 3 and Table 2 represent the yield and the biomass per recruit (*Y/R* and *B/R*) as a function of a multiplier of the fishing effort for both constant and variable *M* values. Thus, a "Factor" of 1 corresponds to the fishing effort currently exerted on the stock (F_c). The current mean Biomass value (*B/R*) per recruit is 12,863g for constant *M* and 8,292g for variable *M*. The biomass gains per recruit for the effort factor recommended by the FAO, namely $F_{0.1}$, compared to the values estimated for *Fc*, is around 2g for constant *M*. In comparison, the gain is almost negligible for variable *M* given the high value of $F_{0.1}$, which is very close to 1 (0.94) (Fig. 3). The values of the current yield-per-recruit (*Y/R*) are around 6.646 g for constant *M* and 3.742 g for variable *M;* these values remain below the maximum balanced production threshold for constant *M* (*MSY* = 6.993 g) as for variable *M* (*MSY* = 4.311 g). Finally, the difference between the number of recruits estimated by the VPA is 340 million more individuals for variable *M*.

Table 3. Yield and biomass parameters of *Sardina pilchardu*s from the central region of the Algerian coast, according to F, for both constant and variable M (obtained by the VIT 1.3 software)

Constant *M*					**Variable *M***				
Factor	**F (yr^{-1})**	Y/R **(g)**	B/R **(g)**	SSB/R **(g)**	**Factor**	**F (yr^{-1})**	Y/R **(g)**	B/R **(g)**	SSB/R **(g)**
F_0 **= 0**	0	0	25.700	20.502	F_0 **= 0**	0	0	14.800	11.202
F_c **= 1**	1.74	6.646	12.863	8.266	F_c **= 1**	1.74	3.742	8.292	5.004
$F_{0.1}$ **= 0.81**	1.41	6.355	14.091	9.397	$F_{0.1}$ **= 0.94**	1.64	3.692	8.460	5.158
F_{MSY} **= 2.9**	5.05	6.993	6.968	4.459	F_{MSY} **= 3.8**	6.53	4.311	4.365	2.619
Number of recruits	**438 188 214.44**				**Number of recruits**	**539 734 212.89**			

F*(yr^{-1})** = fishing mortality; ***Y/R = yield per recruit; ***B/R*** = biomass per recruit; ***SSB/R*** = Spawning stock biomass; F_0 = factor of non-fishing; F_c = factor of current effort fishing; $F_{0.1}$ = factor of fishing mortality rate at which the marginal yield-per-recruit is only 10 percent of the marginal yield-per-recruit on the unexploited

stock; F_{MSY} = factor of fishing mortality rate that maximizes equilibrium yield per recruit; **Number of recruits** = number of exploitable individuals estimated by the VPA.

Results in yield and biomass per recruit estimates as a function of fishing mortality (*F*) (Table 2 ; Fig. 3) reveals a state of "not overfished and not overfishing", for either constant or variable *M* values, according to Froese & Proelss (2012).

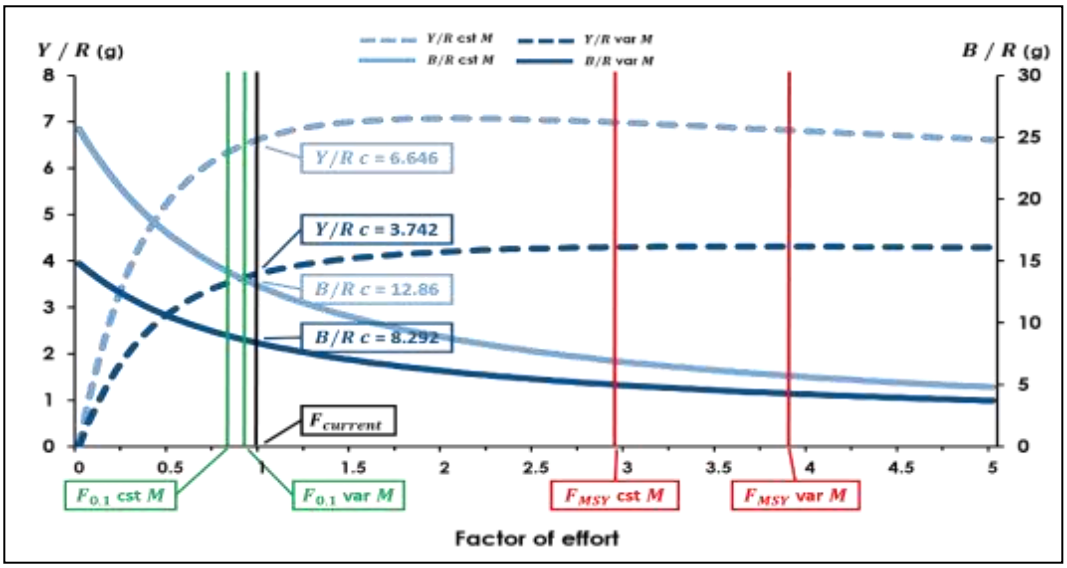


Figure 3. Yields and biomass per recruit of S. pilchardus from the central region of the Algerian coast, according to M.

Biomass and yield in tons. By multiplying the values per recruit of average and fertile biomass, as well as the yield per recruit (g) by the number of recruits (millions), we obtained results in tons (Fig. 4). The biomass gains, for variable *M* compared to constant *M*, are around 13% for the average biomass and 6% for the spawning biomass. For the yield in tons, its value is the same for both constant and variable *M*. This value (2912t) represents the total catch for the year 2018, which is an important input data to run the VPA.

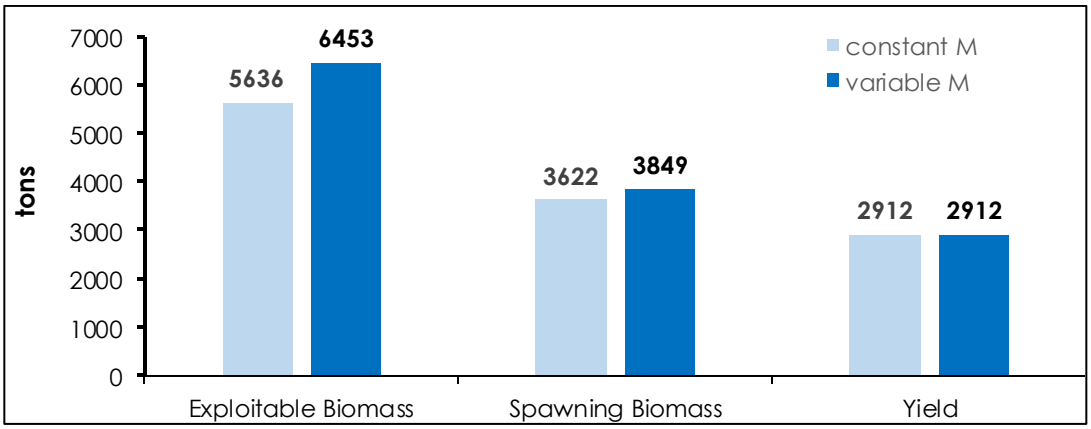


Figure 4. Mean exploitable Biomass, Spawning Biomass and Yield of Sardina pilchardus from the central region of the Algerian coast, estimated in tons for both constant and variable M values by the Vit4win 1.3 software.

However, for Bouaziz et al. (2014) (Tab. 3), the results of the evolution of yield and biomass per recruit as a function of fishing mortality (*F*) in the same region revealed a state of overfishing and overfished. It was recommended in this study to adjust current F_c to $F_{0.1}$,

which represented a 77% reduction in fishing effort in order to allow the maximum sustainable yield in the long-term, whilst ensuring the renewal of the exploitable stock.

Table 4. Results of the VPA for Bouaziz et al. (2014) study of S. pilchardus from the central region of the Algerian coast, for a constant value of M calculated using the Djabali et al. (1994) equation.

Bouaziz et al. 2014 (M= 0.49 yr^{-1})			
Factor	**F (yr^{-1})**	Y/R **(g)**	B/R **(g)**
$F_0 = 0$	0	0	55.47
$F_c = 1$	3.03	10.375	7.387
$F_{0.1} = 0.23$	0.70	11.116	23.137
$F_{MSY} = 0.4$	1.21	11.781	15.538

Conclusion

The use of a natural mortality value (*M*) for each age class has a significant impact on the estimate of exploitable biomass and yield per recruit. It would therefore be recommended to use a value of *M* by age in biomass estimation studies to get closer to reality. Indeed, early ages small pelagic fishes are the most vulnerable classes to mortality caused by predation or pollution induced stress. This fact must be taken into serious consideration for a better monitoring of exploitable stocks, without ignoring the degree of uncertainty of estimates.

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HEAVY METAL ACCUMULATION AND CHEMICAL COMPOSITION OF ESSENTIAL OILS OF HYSSOP (HYSSOPUS OFFICINALIS L.) CULTIVATED ON HEAVY METAL CONTAMINATED SOILS

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Abstract

Comparative research has been conducted to allow us to determine the content of heavy metals and chemical composition of hyssop oils, as well as to identify the possibility of hyssop growth on soils contaminated by heavy metals. The experimental plots were situated at different distances of 0.5 km and 15 km, respectively, from the source of pollution the Non-Ferrous-Metal Works near Plovdiv, Bulgaria. On reaching flowering stage the hyssop plants were gathered. The content of heavy metals in flowering tips of hyssop was determined by ICP. The essential oils of the hyssop were obtained by steam distillation in laboratory conditions which were analyzed for heavy metals and chemical composition was determined. Hyssopus officinalis L. is a plant which is tolerant to heavy metals and can be grown on contaminated soils. Heavy metals do not affect the development of hyssop and the quality and quantity of oil obtained from it. Twenty-two components were identified in the oils. The quantity of identified compounds was shown to correspond to 98.5% and 98.8% of the total oil content. Among the detected compounds, trans-pinocamphone (28,38-29,73%), cis-pinocamphone (19,03-21,37%), germacrene D-4-ol (22,12-24,86%), elemol (5,97-6,28%), and spathulenol (3,08-3,24%) were the major compounds. The results clearly showed that the composition of oil was not affected by soil contamination with heavy metals. The essential oil of Hyssopus officinalis L. can be a valuable product for the farmers from the polluted regions.

Keywords: *Contaminated soils, essential oil composition, heavy metals, hyssop.*

Introduction

Hyssopus officinalis (hyssop) is a species of the genus Hyssopus in the family Lamiaceae. Hyssopus officinalis is distributed in the Mediterranean regions of Europe, grows naturally in southern Europe, the Middle East, Central Asia, North Africa and North America, and is cultivated for culinary, decorative and medicinal purposes in many countries around the world. Hyssop is an herb with antiseptic, toning, antibacterial and antifungal action (Mazzanti et al. 1998, Fraternale et al. 2004), The herb contains many important ingredients, such as essential oils, polyphenols, acids (chlorogenic, n-hydroxybenzoic, protocatechuic, ferulic, feruloylquinic, syringic, vanillic, p-coumaric, rosmarinic, caffeic), flavonoids, polysaccharides, tannins, pigments and resins (Zawislak, 2011).

Hyssop is used as a medicinal, aromatic spice and honey plant. Flowers and leaves are added to a wide range of dishes and are a good substitute for mint. The leaves have a slightly bitter taste due to the tannin content. The flowers are used to flavour green salads, soups, meat. Hyssop is also added to various drinks. It is an indispensable ingredient in the French liqueurs Benedictine and Chartreuse.

The oil is accumulated mainly in the flowering stalks and leaves, while its quantities in the stems are insignificant (Wolski et al. 2006). Hyssop oil is obtained by steam distillation, with extraction yields of about 0.5% in France, Spain, Italy, Germany, Slovenia, Moldova, Bulgaria, Iran, the United States, China and India, with other countries typically producing

less than 5 tons per year. The essential oil of hyssop is a light yellow liquid with grassy, camphor odor with warm and spicy tones and a bitter taste. The oil is used as a fragrance in soaps, perfumes, creams and other cosmetic products (Wesołowska et al., 2010), as well as in aromatherapy (Lis-Balchan, 2006) as a pure essential oil or in combination with other essential oils.

The oil is antimicrobial, mildly antispasmodic and has a strong antiviral effect against HIV (Kaplan et al., 1990). The antibacterial, antifungal and antioxidant properties of hyssop are attributed to the contained pinocamphon, isopinocaphone and β-pinene. The antiviral activity is probably due to the presence of caffeic acid and tannins (Kaplan et al., 1990)

The chemical composition of hyssop oil has been the subject of many studies around the world. In most studies, hyssop oil has been found to be characterized mainly by bicyclic monoterpene ketones, such as cis-pinocamphone and trans-pinocamphone, and by smaller amounts of β-pinene (i.e. the biogenetic precursor of trans-pinocamphone), pinocarvone, limonene, 1,8-cineole, linalool and camphor (Fathiazad et al., 2011). This chemical profile is similar to the results obtained for hyssop oils from Italy (Fraternale et al., 2004), Switzerland (Rey et al., 2004), Hungary (Rey et al., 2004; Németh-Zámbori, 2015), Turkey (Kizil et al., 2010), Finland (Galambosi et al., 2010), Poland (Zawiślak, 2013), Germany (Németh-Zámbori, 2015), Iran (Moghtader, 2014), India (Pandey et al., 2014), Ukraine (Kotyuk, 2015), Bulgaria (Hristova et al., 2015), and Mississippi (Zhelyazkov et al., 2012). However, in hyssop oil in France and Italy has been found to be high in isopinocamphone and linalool (Salvatore et al. 1997), in oil from India - pinocamphone, β-pinene and isopinocamphone (Garg et al., 1999), in oil from Turkey - pinocarvone, pinocamphone, β-pinene, 1,8-cineole and isopinocamphon (Ozer et al., 2005), and in oil from Spain - 1,8-cineole and β-pinene (Garcia-Vallejo et al., 1995).

The composition of the oil depends on various factors, such as genetic origin, habitat, environmental conditions, physiological stage (time of harvest, plant parts of the plant used to extract the essential oil, soil composition, etc.). Due to the strong influence of these factors, the yield and composition of essential oil often do not match the profile defined by ISO 9841: 2013.

It has been found that the medicinal plants can accumulate larger amounts of heavy metals such as Cd, As, Pb and Hg compared to other plants (Kabata Pendias and Pendias, 2011). The plants of the Lamiaceae family are also widespread in world and have a large biomass production capacity. However, there are no studies on the possibilities of heavy metals accumulation in Hyssopus officinalic L. when grown on contaminated soils. There are no comprehensive studies on the relationship between the total content of heavy metals in the soil, their uptake by the flowering tips of hyssop and quality of oil.

The purpose of this work is to conduct a comparative study, which allows us to determine the heavy metal accumulation in hyssop, the quality of hyssop oil, as well as the possibilities to grown on heavy metal contaminated soils.

Material and Methods

The experiment was performed on an agricultural fields contaminated by Zn, Pb and Cd, situated at different distances (0.5, and 15.0 km) from the source of pollution, the NFMW near Plovdiv (Non-Ferrous Metal Works), Bulgaria.

Characteristics of soils are shown in Table I. The soils were slightly neutral to alkalic with moderate content of organic matter and essential nutrients (N, P and K) (Table I). The psedo-total content of Zn, Pb and Cd is high and exceeds the maximum permissible concentrations (MPC) in soil 1 (S1) (Table 1).

Table 1.Characterization of the soils

Parameter	pH	EC, dS/m	Organic C,%	N Kjeldal,%	P, mg/kg	K, mg/kg	Pb, mg/kg	Zn, mg/kg	Cd, mg/kg
Soil 1 (S1) 0,5 km	7,4	0,15	2,2	0,34	625,6	6960	2509,1	2423,9	64,3
Soil 2 (S2) 15 km	7,5	0,15	1,54	0,12	387,3	6780	49,4	172,7	1

MPC (pH 6.0-7.4) – Pb-100 mg/kg, Cd-2.0 mg.kg, Zn-320 mg/kg
MPC (pH >7.4) – Pb-100 mg/kg, Cd–3.0 mg/kg, Zn-400 mg/kg

The test plant was hyssop. Hyssop is grown according to conventional technology. Five plants of each of the areas were used for the analysis. Upon reaching the stage of flowering, hyssop was harvested and the content of Pb, Zn and Cd in flowering tips was determined. The essential oil of the hyssop was obtained by steam distillation in laboratory conditions which was analyzed for heavy metals and its chemical composition was determined.
Pseudo-total content of metals in soils was determined in accordance with ISO 11466. The available (mobile) heavy metals contents were extracted in accordance with ISO 14870 by a solution of DTPA. The contents of heavy metals (Pb, Zn and Cd) in the plant material (leaves) and in the essential oils of hyssop were determined by the method of the microwave mineralization. The quantitative measures were carried out by ICP method (Jobin Yvon Emission - JY 38 S, France). Digestion and analytical efficiency of ICP was validated using a standard reference material of apple leaves (SRM 1515, National Institute of Standards and Technology, NIST).
The chemical composition of the oil was determined on a gas chromatograph PYE UNICAM series 204, equipped with a flame ionization detector and a capillary column CARBOWAX 20 M with hydrogen carrier gas.

Results and Discussion

Soils
The results presented in Tables I and II show that In the soil samples S1 (taken from the area situated at the distance of 0.5 km from NFMW), the reported values for Pb were exceeding MPC approved for Bulgaria and reached to 2509.1 mg/kg. In the area located at a distance of 15 km, the contents of Pb significantly reduce to 49.4 mg/kg. Similar results were obtained for Cd and Zn. The results for the mobile forms of the metals extracted by DTPA show that the mobile forms of Cd in the contaminated soils are the most significant portion of its total content and reached to 57,2%, followed by Pb with 33,8 % and Zn with 9,8%.
In the soil located at a distance of 15 km from NFMW the mobile forms of Cd are the most significant part of its.

Table 2. DTPA-extactable Pb, Zn and Cd (mg/kg) in soils sampled from NFMW

Soils	Pb		Cd		Zn	
	mg/kg	%*	mg/kg	%	mg/kg	%
S1	849.1	33.8	36.8	57.2	236.8	9.8
S2	21.5	43.5	0.7	70	38.9	22.5

*DTPA -extractable / total content

Content of Heavy Metals in hyssop

A significant accumulation of Pb is found in the flowering tips of the hyssop. The content of this element reaches up to 33.6 mg/kg in leaves of the hyssop grown at a distance of 0.5 km from NFMW (Table 3). The content of Cd in the flowering tips of hyssop grown at a distance of 0.5 km from NFMW reaches up to 1.67 mg/kg, values considered to be non toxic to plants. According to Kabata-Pendias and Pendias (2011) 5.0 mg/kg Cd is considered to be a toxic value for the plants. The content of Zn in the flowering tips of hyssop grown at a distance of 0.5 km from NFMW reaches up to 69.3 mg/kg, as these values are also less the critical values for plants - 100-400 mg/kg. Probably a portion of heavy metals absorbed by the conduction system moves from the roots to the above-ground parts of the hyssop and are accumulated predominantly there. With increasing the distance from NFMW a clear trend is seen towards reducing the content of heavy metals in the flowering tips of the studied crop. The content of heavy metals in the flowering tips of the hyssop grown at 15 km from NFMW reaches up to 1.06 mg/kg Pb, 0.02 mg/kg Cd and 15.2 mg/kg Zn.

Table 3. Content of Pb, Cd and Zn (mg/kg) in flowering tips and essential oil of hyssop

Soils	Pb		Cd		Zn	
	Flowering tips	oil	Flowering tips	oil	Flowering tips	oil
S1 (0.5 km)	33.6	0.08	1.67	nd	69.3	1.5
S2 (15.0 km)	1.06	0.05	0.02	nd	15.2	1.1

n.d.-non detectable

The heavy metal content in the essential oil from hyssop was also determined. The results obtained show that the majority of the heavy metals contained in the flowering tips of the hyssop do not pass into the oil during the distillation, therefore their content in the oil is much lower. Pb content in the essential oil of hyssop reaches up to 0.08 mg/kg, Zn up to 1.5 mg/kg, while the content of Cd is below the limits of the quantitative measurement of the method used. Significantly lower are the figures in the essential oil of hyssop grown at a distance of 15 km from NFMW – 0.05 mg/kg Pb and 1.1 mg/kg Zn. The results obtained show that the content of heavy metals in the essential oils is much lower compared to the flowering tips of the hyssopus, and the amounts of Pb, Zn and Cd in the oil of hyssop are lower than the accepted maximum values and meet the requirements of an environmentally friendly product.

Effect of heavy metals on the quality of the oil

The results of the chromatographic analysis of essential oils obtained by processing of leaves of hyssop grown at a different distance from NFMW are presented in Table 4. The values of the main components of the essential oil of hyssop are compared with the requirements of ISO 9841:2013 for the hyssop oil. ISO 9841:2013 regulates the amounts of the following constituents in the essential oil: sabinene (1,0-3,5%), trans-pinocamphone (25,0-45,0%), cis-pinocamphone (8,0- 25,0%), β-caryophyllene (1,0-3,0%), germacrene D (1,2-4,5%), elemol (0,2 - 2,5%), spathulenol (0,1-1,5%).

The yield for hyssop oils ranged between 0.50% (S1) and 0.55%.(S2). According to literary data, the oil content in fresh hyssop varies from 0.03-0.16% (Schulz and Stahl-Biskup, 1991; Khazaie et al., 2008), from 0.13% to 0.26% (mean of 0.19%) (Zhelyazkov et al., 2012), 0.25% Garg et al. (1999), 0.65 - 0.75% in blue hyssop, 0.7-1.1% in pink hyssop, 0.6-1.0% in white hyssop (Chalchat et al., 2001, Wesolowska et al., 2019).

22 compounds representing 98.53-98.81% of the oil were identified, with seven monoterpene hydrocarbons (5.303-6.587%), five oxygen-containing monoterpenes (50.74-54.19%), one phenylpropanoid (0.50-0.53%) and five oxygen-containing sesquiterpenes (29.07-31.47%).

The obtained results show that oxygen-containing monoterpenes (trans-pinocamphone, cis-pinocamphone, beta-linalool, terpin-4-ol and myrtenol) predominate in the oil, with no significant differences in the composition between the oils obtained from the areas of different distance from NFMW; the content of beta-linalool varies from 0.641% in the contaminated area (S1) to 0.674% in the uncontaminated area (S2), trans-pinocamphone from 28.376% (S1) to 29.728% (S2), cis-pinocamphone from 19.028% (S1) to 21.371% (S2), terpin-4-ol from 1.362% (S2) to 1.694% (S1) and myrtenol from 0.999% (S1) to 1.052% (S2).

Table 4. Composition of oil of hyssop (%) obtained by processing fresh flowering tips of hyssopus

№	Compound	RI	S1 (0.5 km)	S2(15.0 km)	ISO 9841
			% of TIC		min-max
1	Camphene	953	0.263	0.250	
2	Sabinene	969	1.554	2.426	1.0-3.5
3	β-Myrcene	991	0.998	1.548	
4	α-Phellandrene	1005	0.452	0.429	
5	p-Cymene	1024	0.425	0.403	
6	β-Phellandrene	1031	0.554	0.527	
7	beta-Linalool	1097	0.674	0.641	
8	trans-Pinocamphone	1160	29.728	28.376	
9	cis-Pinocamphone	1173	21.371	19.028	25.0-45.0
10	Terpin-4-ol	1177	1.362	1.694	8.0-25.0
11	Myrtenol	1194	1.052	0.999	
12	Methyleugenol	1401	0.527	0.501	
13	β-Caryophyllene	1419	1.581	1.502	1.0-3.0
14	α-Humulene	1454	1.057	1.004	
15	Germacrene D	1480	1.852	1.760	1.2-4.5
16	Elemol	1549	6.284	5.970	0.2-2.5
17	Germacrene D-4-ol	1575	22.115	24.859	
18	(-)-Spathulenol	1578	3.240	3.078	0.1-1.5
19	Caryophyllene oxide	1581	1.987	1.887	
20	α-Bisabolol	1683	0.791	0.751	
21	Farnesol	1712	0.939	0.892	
22	Camphene	953	0.263	0.250	
	Total		98.805	98.525	

RI - Relative Index; TIC - Total Ion Current

The oils from the contaminated area (S1) have a higher content of germacrene D-4-ol (oxygen-containing sesquiterpene), and a lower content of spathulenol, caryophyllene oxide, α-bisabolol and farnesol compared to the oil from the uncontaminated area (S2). Our results show that the dominant ingredients in hyssop oils are trans-pinocamphone and cis-pinocamphone, which is in accordance with the results obtained by Mitić and Dordevic (2000), Fraternale et al. (2003), Rosaon et al. (2002), Rey et al. (2004) and Zhelyazkov et al. (2012). It is noteworthy that the content of trans-pinocamphone prevails over cis-pinocamphone, as the reported values are lower in the oils from the contaminated area. The content of trans-pinocamphone varies from 28.376 (S1) to 29.728% (S2) and the values are within the ISO 9841 (25.0-45.0%). The cis-pinocamphone content ranges from 19.028% (S1) to 21.371% (S2) and the values are within the ISO 9841 (8.0-25.0%).

In some studies, cis-pinocamphone has been found to predominate over trans-pinocamphone (Mazzanti et al. 1998, Baj et al. 2010, Wesołowska et al. 2010), while in others it has been found that trans-pinocamphone is the dominant compound (47.9–51.4%) (Kizil et al., 2008, 2010) and the difference between these two components can reach up to three times. Mitić and Dordevic (2000) found a content of 44.7% of cis-pinocamphone and 14.1% of trans-pinocaphone, while Rey et al (2004) found 40-60% of trans-pinocamphone and 20–30% of cis-pinocamphone. Similar are the results obtained by Garg et al. (1999), according to whom hyssop oil contains significantly more trans-pinocamphone (49.11%) than cis-pinocamphone (9.69%). However, the results obtained by Zawislak (2013) for hyssop oil from Poland show a smaller difference between the content of cis-pinocaphone (33.52-37.13%) and trans-pinocamphone (23.43-28.67%),

A significant content of germacrene D-4-ol has also been found in the oils we studied, the amount of which varies from 22.115% (S2) to 24.859% (S1). A review of the literature shows that most hyssop oils do not contain germacrene D-4-ol. Germacrene D-4-ol has been found to be present in Serbian oils, but its amount is significantly lower (5.7%) (Mitić and Dordević, 2000).

No β-pinene has been found in the studied oils, similar to the oils from Serbia. Hyssop oils from Italy, India and Hungary are reported to be rich in β-pinene: Italy (11.15% and 10.5–10.8%) (Mazzanti et al. 1998, Fraternale et al. 2004), India (19.4%) (Garg et al. 1999) and Hungary (4–15%) (Rey et al. 2004), while the oil from Poland contains a very small amount of β-pinene (0.2%) (Wesołowska et al. 2010). It has been found that the content of β-pinene in the oil varies depending on the stage of plant growth, with the largest amount of β-pinene in hyssop oil in the vegetative stage (9.9-13.9%)(Zawislak, 2013). In a study by Rosaon et al. (2002), however, the content of β-pinene in the oil isolated from the raw material extracted in the full flowering phase is higher and reaches up to 14.88% and 15.07%.

Pinenes and bicyclic terpenes can be found in the essential oils of conifers (pines), rosemary, lavender and turpentine (da Silva et al., 2012). β-pinene is considered one of the most bioactive components in the essential oils of the family Lamiaceae. Pinenes show antimicrobial and fungicidal activity (da Silva et al., 2012) and have been used for centuries for the production of fragrances.

The content of germacrene D varies from 1.760% (S1) to 1.852% (S2) and the values obtained are within the ISO 9841 range (1.2-4.5). According to Zawislak (2013), the content of germacrene D varies from (1.4–4.5%) to (5.0–6.2%), with the lowest amount of this compound in the hyssop oil from full flowering. The oil from Poland has been found to contain germacrene D (3.23-4.65%), while the oils from India (Garg et al. 1999) and Serbia (Mitić and Dordević 2000) have been found to contain small amounts of germacrene D, 0.65% and 1.6%, respectively. The content of elemol in the oils varies from 5.970% (S1) to 6.284% (S2) and exceeds the values specified in the standard (0.2-2.5). According to Zawislak (2013), the content of elemol varies from 0.3 to 6.5% in hyssop oil, with the highest content in the oil of full-flowering plants. Similar to values present in this study are those found by Mitić and Dordević [2000] (5.6%) and Baj et al. [2010] - (7.4%). Significantly higher values have been found by Wesołowska et al. (2010) (17.21%), while Mazzati et al. (1998) has found a very low content (1.7%). Grag et al. (1999), Rosaon et al. (2002) and Fraternale et al. (2004) have not detected the presence of elemol in hyssop oil.

Exceeding the standard ISO 9841 is also observed in the tricyclic sesquiterpene alcohol spathulenol, which contributes to the earthy herbal fruity aroma of the oil. Its content varies from 3.078 (1) to 3.240% (2) (ISO 9841 (0.1-1.5%) High values of spathulenol (2.14%) are found in oils in Iran and significantly lower for oils from Poland (0.6-1.0%)

The content of sabinene in the studied oils varies from 1.554 (2) to 2.426 (1) and is within ISO 9841 (1.0-3.5%). Similar results have been found for the oils from Moldova (1.31%)

(Goncearіuc and Balmus, 2013), and Bulgarian commercial oil (2%)(Stoyanova and Grozeva (2006).

The content of methyleugenol in the oils we studied varies from 0.501% (S1) to 0.527% (S2). Similar results are obtained for oils from Serbia (0.4%), while in the oils from Montenegro methyleugenol is the main component (38.3%) (Gorunovic et al., 1995).

According to Garcia-Vallejo et al. (1995) 1,8-cineole is the main component in the oil from Spain (52.9%). However, no 1,8-cineole has been found in the studied oils. Similar results have been obtained by Tsankova and Kontaktchiev (1993) for commercially produced oils in Bulgaria, as well as for oils from Mitic and Đorđevic (2000).

So far, eight groups of chemotypes have been identified, based on the main components (more than 5%) contained in hyssop oil: (1) oils with abundance of pinocarvone; (2) oils which are rich in pinocamphone; (3) oils with higher proportions of isopinocamphone; (4) oils containing large amount of linalool; (5) 1.8-Cineole rich oils, (6) oils containing large amount of linalool, (7) oil rich in methyl eugenol and (8) α-Pinene rich oil (Ogunwande et al., 2011).

The results of this study show that hyssop oil belongs to the trans pinocamphone type of oil: trans-pinocamphone (28.376-29.728%) > germacrene D-4-ol (22.115-24.859%) > cis-pinocamphone (19.028-21.371%) > elemol (5.970-6.284%). The studied oils are a new subtype of the trans-pinocamphone type of oils. So far, in the scientific literature there is no information about such a subtype of trans-pinocamphone type of oils. In Bulgaria, the studies about commercially produced oil shows that the oil belongs to the pinocamphone type of oils: cis-pinocamphone (48.98 - 50.77%), β-pinene (13.38 - 13.54%), trans- pinocamphone (5.78) - 5.94%) and β-phellandrene (4.44 - 5.17%)(Hristova et al., 2015); iso-pinocamphone (40.2%), pinocaphone (10.3%) and β-pinene (14.2%) (Tsankova and Kontaktchiev, 1993), while Stoyanova and Grozeva (2006) found pinocamphone (38.4–46.5%), β-pinene (9.2–14.2%), 1.8-cineole (9.0–12.0%) and camphor (8.0–12.5%).

The results from present study confirm that hyssop plants have a unique chemical composition and individual oil chemotype.

The chromatographic profile shows a complex mixture of components contained in hyssop oil. Figure 1 shows the classification of the identified compounds on the basis of functional groups. The highest is the content of oxygen-containing monoterpenes (50.74-54.19%), followed by oxygen-containing sesquiterpenes (29.07-31.47%), monoterpene hydrocarbons (5.30-6.59 %), and phenipropanoid (0.50-0.53%).

Zawislak (2013) have found that monoterpene ketones have the highest percentage in hyssop oils (62%), followed by monoterpene hydrocarbons (20.9–22.2%) in vegetation plants and (7.1–13.3%) in full flowering plants, and sesquiterpenes hydrocarbons (10.1–11.8%) in plants harvested at the beginning of the flowering phase. Similar results have been obtained by Hristova et al. (2015) for oil originating in Bulgaria (oxygen-containing monoterpenes (61.69%), monoterpene hydrocarbons (20.77%) and sesquiterpene hydrocarbons) (15.19%). There are no significant differences in the profile of essential oils extracted from hyssop when grown on contaminated and uncontaminated soils. Probably the contamination of the soil with heavy metals does not affect the composition and quality of the oil. The content of the main components contained in hyssop oil grown on contaminated and uncontaminated soils is within the standard, with the exception of elemol and spathulenol, which exceed the corresponding value in the ISO 9841.

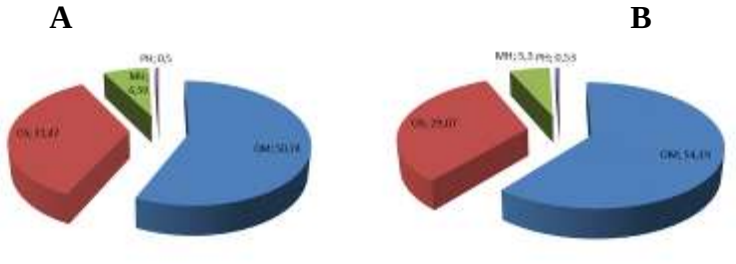


Figure 1. Classification of the identified compounds based on functional groups in oils from contaminated soils (A) andnon-contamonated soils (B)(OM - oxygenated monoterpenes; MH - monoterpenic hydrocarbons; OS – oxygenated sesquiterpenes;; PH - phenipropanoid)

This study shows that hyssop can be grown on heavy metal contaminated soils. The oil yield, essential oil content and essential oil compositions are similar to the oil from the uncontaminated area and to the oils currently available on the market from other countries. Therefore, essential oils extracted from hyssop grown in contaminated areas could be marketed in the same way as essential oils produced in other regions of the world.

Conclusions

Based on the obtained results, the following conclusions can be made:

1. Hyssopus oficinalis L. is a plant which is tolerant to heavy metals and can be grown on contaminated soils.
2. The amounts of Pb, Zn and Cd in the oil of hyssop grown on contaminated soil (Pb -2509.1 mg/kg, Zn -2423.9 mg.kg, Cd - 64.3 mg/kg) are lower than the accepted maximum values and meet the requirements of an environmentally friendly product.
3. The contamination of the soils with heavy metals does not affect the composition and quality of the oil. The content of the main components contained in hyssop oil grown on contaminated and uncontaminated soils is within the ISO 9841, with the exception of elemol and spathulenol, which exceed the corresponding value in the ISO 9841.
4. The highest is the content of oxygen-containing monoterpenes (50.74-54.19%) in essential oils of hyssop, followed by oxygen-containing sesquiterpenes (29.07-31.47%), monoterpene hydrocarbons (5.30-6.59 %), and phenipropanoid (0.50-0.53%).
5. Hyssop oil from contaminated and uncontaminated area belongs to the new subtype of the trans-pinocamphone type of oils (trans-pinocamphone (28.376-29.728%) > germacreneD-4-ol(22.115-24.859%) > cis-pinocamphone(19.028-21.371%) > elemol (5.970-6.284%).
6. The essential oil of hyssop can be a valuable product for the farmers from the polluted areas.

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THE EFFECT OF NONIONIC SURFACTANTS ON CADMIUM (II) REMOVAL RATE USING BULK LIQUID MEMBRANE SYSTEM

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Abstract

Industrial and agricultural wastewater is a significant source of heavy metal pollution. Application of different membrane systems for selective removal of heavy metal ions from natural resources is very interesting in recent years. One of the most efficient applications of these systems is based on the implementation of the "bulk liquid membrane" (BLM), which includes a combination of three processes: extraction, diffusion, and re-extraction of an analyte. In this paper, the removal of cadmium (II) ions through a liquid membrane system and factors that influence the process were examined. The research was performed using the homemade transport cell. Metal ion concentration in aqueous phases was monitored by flame atomic absorption spectrophotometry, after 3 hours of experiment. Macrocyclic ethers (18-crown-6, benzo-18-crown-6, dibenzo-18-crown-6, dicyclohexane-18-crown-6), were used as ligands for Cd(II) ions. The effects of nonionic surfactants (Triton X-100, Brij 35, Brij 58 and Brij 78) as additional possible carriers within the liquid membrane were investigated. The results showed that surfactants reduced the transport rate, but significant removal of cations from source phase occurred (64% Cd(II) removed using Brij 35 surfactant).

Keywords: *Cd(II) removal, Bulk liquid membrane transport, Nonionic surfactants*

Introduction

In recent years, heavy metal pollution has become a worldwide problem due to their highly toxic potential and tendency to accumulate in the body. Therefore, the research of interactions within analytical systems, finding the most efficient and reliable procedures for quantitative determination and improvement of affordable methods for wastewater treatment has recently been the subject of research by many researchers around the world. Transport through the liquid organic membranes (bulk liquid membranes, BLM) has lately attracted the particular attention of researchers. The main features of this technology are its low start-up and operating costs, low carrier, solvent and energy consumption, the easy capability of commercialization, and high selectivity (Alguacil, 2002). Liquid membrane is known as a green technology because of its green characteristics such as being eco-friendly and its low consumption of organic solvent (Chang et al., 2010). The implementation of the "bulk liquid membrane" BLM system is a relatively simple process and required the construction of transport cells with two aqueous phases: the source phase (contained metal ions that need to be transported) and the receiving phase (in which the ions will be transported). The aqueous phases were separated from the organic phase (the membrane that contained the ligand dissolved in a suitable solvent).

Polyether ligands are among the most suitable host molecules for many metal ions, due to the presence of oxygen atoms as electron donors in their structure, which enables the formation of a coordination-covalent bond. The ligand-cation (″host-guest″) interactions and the stability of the formed complexes depend on a number of experimental conditions (Salman et al.,

1996). Important parameters are the type of membrane solvent, the type of counter-ion, the presence of coexisting species, stripping agents, etc. Since the ligands are the "carriers" of metal ions through the membrane, appropriate values of the stability constants of their complexes, adequate solubility within the membrane, as well as lipophilicity are required to prevent the ligand from leaking out of the membrane into the solution of the analyte itself. The nature of the membrane solvent is also one of the main factors in establishing transport efficiency. The physical and chemical properties of solvents are closely related to their structures and play important roles in solute-solvent and solute-solute interactions in solutions. Researchers (Izatt et al., 1991) highlight the necessity of having valid information for different solvent systems to understand their influence on thermodynamics and complexation kinetics.

Authors (Malihe et al., 2010) assumed that the most influential process during transport experiments was the metal ions released from the complex in the membrane phase to the receiving aqueous phase through the contact surface between the two phases. Based on this assumption, authors also proposed a possible mechanism for metal ion transport (Figure 1.).

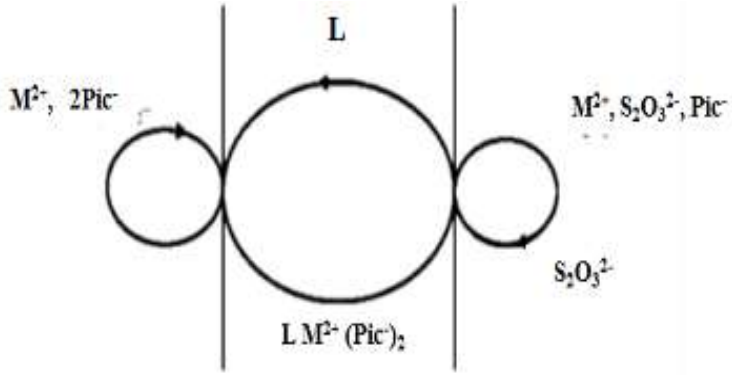


Figure 1. Proposed mechanism of metal ion transport through an organic liquid membrane containing dissolved ligand (L), from SP (contains: M^{2+} cations and counter ions picrate Pic$^-$) to RP (contains stripping thiosulphate ions)

In this paper, ″model systems″ with known concentrations of Cd (II) ions and macrocyclic ligands (18-crown-6, benzo-18-crown-6, dibenzo-18-crown-6, dicyclohexano-18-crown-6) were examined with counter ions (picrate) present, in dichloromethane as an organic solvent. The effect of adding nonionic surfactans (also polyether compounds) as possible carriers is presented as metal ion transport efficiency, expressed through the metal ion concentration in aqueous phases of the system, and measured with atomic absorption spectrometric (AAS) technique.

Material and Methods

For every transport experiment, two aqueous solutions and one non-aqueous organic solution (liquid membrane), were prepared, as follows.

Source Phase solutions were prepared using the AAS standard cadmium (II) solution from Merck and picric acid (99%, Kemika) and adjusted to pH = 5, using the acetic buffer solution. Membrane Phase solutions were prepared by dissolving different macrocyclic ligands: 18-crown-6, 18C6; benzo-18-crown-6, B18C6; dibenzo-18-crown-6, DB18C6; dicyclohexano-18-crown-6, DCH18C6 (99%, ACROS ORGANICS) and non-ionic surfactants: TX-100, Brij 35, Brij 58, Brij 78 (p.a. Sigma-Aldrich) in organic solvent (p.a. Kemika) dichloromethane (DCM). Receiving Phase solutions were prepared also in acetic buffer medium (pH = 5), by dissolving sodium thiosulphate (p.a. Sigma-Aldrich) in it.

Transport Procedure

Cylindric glass container, i.e. "transport cell", with inner diameter of 5 cm and central glass tube (2 cm in diameter), have been used for this study (Figure 2.).

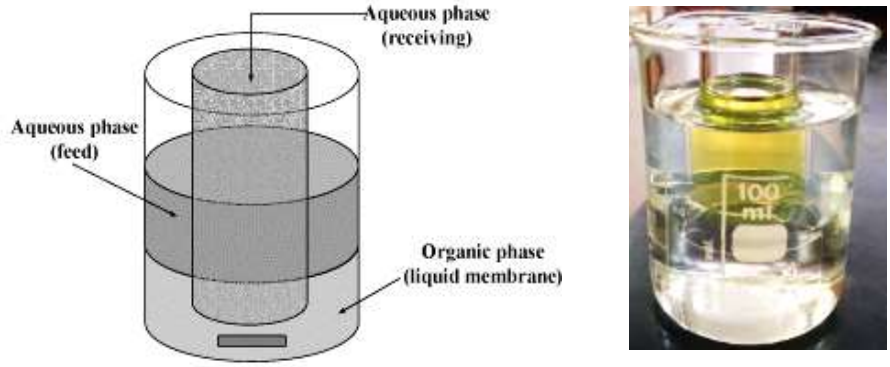


Figure 2. Scheme of a cylindrical glass vessel, "transport cell"

Central tube provides physical separation of two aqueous phases: source phase (SP), and receiving phase (RP). Membrane phase (MP) lies under the aqueous phases and connects them. Constant stirring with a magnetic stirrer is essential to provide that contact surfaces: RP/MP/SP remains flat and well defined.

The source phase (SP) contained 10 mL of a mixture of tested metal ion ($1 \cdot 10^{-3}$mol/L) and the counter ion, picrate ($1 \cdot 10^{-3}$mol/L). The receiving phase (RP), which is outside the central tube, contained a stripping agent (thiosulphate). The membrane phase (MP) contained 50 mL of a suitable ligand ($1 \cdot 10^{-3}$ mol/L) dissolved in an organic solvent; the membrane layer lies beneath the aqueous phases and connects them. The membrane layer lies beneath the aqueous phases and connects them. The membrane phase is mixed with a magnetic stirrer so that under these conditions the contact surfaces between the aqueous phases are straight and precisely defined (Nipamanjari et al., 2010). Both aqueous phases were analyzed by atomic absorption spectrometry after 3 hours, and the concentration of metal ions transported through the membrane was measured.

Instruments

pH measurements of aqueous solutions were performed using the pH meter (GLP31 Crison Instruments). Quantification of metal ions removed during the transport experiments was obtained by Flame Atomic Absorption Spectrometry technique, using the instrument Perkin Elmer AAnalyst 200.

Results and Discussion

Since in our previous investigations of BLM systems (Suljkanović et al., 2017), dichloromethane liquid membrane showed higher transport efficiency compared to 1,2-dichloroethane (48% for Cd (II) and 70.40% for Pb (II)), in this investigation the same organic solvent was used as a liquid membrane for Cd (II) removal. The influence of substituents in structure of macrocyclic ligands, derived from 18-crown-6, reflected in a decreased amount of transported Pb(II) and Cd(II) ions, compared to 18-crown-6, in all used solvents, following the order: DCH18C6<DB18C6<B18C6<18C6 (Suljkanović et al., 2020).

In this research, the interactions of nonionic surfactants from the group of polyethoxylated alcohols (Brij 35, Brij 58 and Brij 78) with Cd(II) ions were investigated, during the transport experiments followed by Cd(II) complexation with macrocyclic ligands within DCM liquid membrane. Results show lower transport efficiencies for systems with added surfactants,

compared to systems contained only macrocyclic ligands (Table 1.). Influence of surfactant structure is obvious: comparation of the results of Cd(II) transport confirms the fact that the the length of the polyether chain affects the metal-surfactant interactions. Despite the assumption that the spatial separation of cations from longer chain surfactant binding sites reduces the level of interactions, the greater number of binding sites (oxygen atoms) in the longer chain surfactant structure speaks in favor of a greater possibility of interactions. Results showed that reducing the number of oxyethylene units in the structure of surfactants leads to a reduction in the content of transported ions, as follows: Brij 35 > Brij 78 > Brij 58. Although the transport of cations to the RP decreases, compared to systems without surfactants, the uptake from the SP is evident for all used surfactants. Formation of reverse micellar structures within the organic membrane can be possible explanation of this phenomenon.

Table 1. Measured content of Cd(II) ions in aqueous phases of BLM system after 3h of transport for different crown ethers and nonionic surfactants within DCM membrane

Dichloromethane membrane	% Cd(II)			
Carrier(s)	SP	MP	RP	Removal
18C6	27	24.9	48.1	73
18C6/TX-100	40	2.5	57.5	60
18C6/Brij 35	36	17.6	46.4	64
18C6/Brij 78	63	6.6	30.4	37
18C6/Brij 58	70	21.9	8.1	30
B18C6	51	9	40	49
B18C6/TX-100	61	1.4	37.6	39
B18C6/Brij 35	60	13.2	26.8	40
B18C6/Brij 78	70	20.9	9.1	30
B18C6/Brij 58	73	19.7	7.3	27
DB18C6	60	1.7	38.3	40
DB18C6/TX-100	70.4	7.3	22.3	29.6
DB18C6/Brij 35	69	10.5	20.5	31
DB18C6/Brij 78	69.1	11.9	19	30.9
DB18C6/Brij 58	85	10.5	4.5	15
DCH18C6	55	20	25	45
DCH18C6/TX-100	68	1.1	30.9	32
DCH18C6/ Brij 35	66	9.5	24.5	34
DCH18C6/ Brij 78	61	1.3	37.7	39
DCH18C6/Brij 58	70	5	25	30

In earlier investigations, nonionic surfactant Triton X-100 was tested as a possible carrier in the membrane (without macrocycle) and after the first 4 hours of the experiment, there was no obvious transport of Cd (II) ions into the receiving phase, although the uptake from source phase was significant (Suljkanović et al., 2014). This confirms possible interactions between metal ions and nonionic surfactants, but those interactions do not provide the required equilibrium between complexation and decomplexation which is the essence of transport processes. It can be assumed that micellar aggregates formed in membrane phase (reverse micelles) incorporate metal ions in their structure, so they can not be released into the receiving phase. The same order of Cd(II) transport rate for used macrocyclic ligands

(Suljkanović et al., 2020) is evident in these surfactant systems only in case of Triton X-100 and Brij 35 surfactants: DCH18C6<DB18C6<B18C6<18C6.
The presence of benzo- and cyclohexyl- groups probably reduce interactions between these substituted macrocycles and cations, which lead to a lower transport rate. Slightly higher transport rate for B18C6 compared to DB18C6 is probably related to higher rigidity of double substituted DB18C6 ring (Amini et al., 1992). Actually, DB18C6 has the most rigid structure among the crown ethers studied (Kashanian et al., 1989). Also, the addition of two benzo groups (as in DB18C6), caused the value of complex stability constant to decrease due to the loss of the flexibility of the ring, resulting with electron withdrawal from oxygen atoms, which consequently reduces the interactions between cations and ligands, lowering the transport efficiency. On the other hand, for DC18C6, the presence of two cyclohexyl groups causes an increase in the basicity of the oxygen atoms in comparison with DB18C6 which leads to the ability of the DC18C6 complex to adapt to different conformations as required for greater stability of the complex (Salman et al., 1996).

Conclusions

The presence of nonionic surfactants Brij 35, Brij 78 and Brij 58 with macrocyclic ligand in liquid membrane decreases transport efficiency due to incorporation of formed complexes into the micellar structures (reverse micelles) which stabilize them, preventing decomplexation and metal ions releasing from membrane to receiving phase. Significant interactions between cations and nonionic surfactants inside the membrane phase of the transport system, lead to certain removal of cations (up to 64% Cd(II) with Brij 35/18C6 in dichloromethane) from the source phase into the membrane phase.

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ECO-FRIENDLY METHOD FOR PRETREATMENT OF LIGNOCELLULOSIC TEXTILES

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Abstract

Natural fibres fabrics are subjected to a pretreatment process to remove cellulose attendants such as: pectin, hemicelluloses, waxes, etc. The world current trends for eco-friendly industrial procedure can be successfully applied also in textile industry. The aim of our research was to study the efficiency of an eco-friendly complexing agent (sodium citrate) and ultrasound energy in enzymatic pretreatment (bioscouring) of a 30% of hemp + 70% of cotton material. The treatment was carried out in an aqueous solution containing a commercial pectinases mixture (Beisol PRO), 2 g/L sodium citrate and 0.5% washing agent (Denimcol Wash RGN). The hydrolytic reaction was developed at 55^0C, the liquid to fabric ratio being 20:1. In addition to the use of less environmentally aggressive chemicals, the proposed scouring treatment used lower reaction temperatures and shorter lead times compared to the classical method. Improvement of the process and better properties for treated materials were obtained by intensifying the effect of the enzyme mixture through the ultrasonic reaction bath at a frequency of 45 kHz. In order to determine the new properties of the bioscoured fabrics, weight loss, hydrophilicity, tensile strength, elongation at break, CIELAB colour system analysis of the dyed samples with alizarin and ruthenium red dyes were performed. The results showed that the eco-friendly method performed in the presence of sodium citrate and ultrasound was effective at a lower enzyme concentration and a shorter treatment time, thereby reducing the costs and the possibility of degradation of the treated material.

Keywords: *Bioscouring, eco-friendly complexing agent, hemp-cotton fabrics, commercial enzyme product, ultrasound*

Introduction

The non-cellulosic attendants of the studied material (hemp/cotton) such as pectin and lignin diminish the quality of these fibres by decreasing the hydrophilic character as well as by the lack of colour uniformity on the entire surface of the material, in case of dyeing. These are the reasons that make it necessary to find suitable methods for removing the mentioned attendants, paying particular attention to the aspects regarding pollution and efficiency of the chosen procedure. The classic method of scouring (removal of non-cellulosic attendants) consists of treating the material with concentrated solution of NaOH at boiling temperature. This treatment destroys not only non-cellulosic carriers but also partially cellulose. As a result of this treatment, high water values of CCO and COB are obtained (Traore, 2000). The elimination reaction of the non-cellulosic components by NaOH treatment is a hydrolytic reaction where the cellulose intermolecular bonds β 1-4 are broken and the polymer is fragmented into smaller chains with lower mechanical properties. An eco-friendly solution for scouring is the treatment with enzymes that preferentially destroy pectin and not cellulose bonds. The enzymes used to remove pectins are mainly pectinases being specific to the respective substrate (Csiszár, 2001). Pectinases are a group of enzymes that fragment pectins.

They behave in nature as carbon recycling agents, degrading the pectin substances to saturated or unsaturated galacturonans, which are further converted by degradation into 5-keto-4-deoxy-uronic acid and finally to pyruvic acid and glycerinaldehyde (Frandsen, 1994; Voragen, 2009). Enzymatic treatment for non-cellulosic compounds removal is done usually with a complex of enzymes system consisting mainly of pectinases and pectinesterases which catalyze the methyl ester hydrolysis, respectively, exo- and endo-polygalacturonases which break the 1–4 carbohydrate bonds (Calafell, 2004). Besides the specific enzyme for pectin removal, the bioscouring solution contains wetting substances and complexing agents like EDTA (ethylenediaminetetraacetic acid). This complexing agent is the most used because complexes with the majority of the metal ions and has a good stability in alkaline medium. Despite its effectiveness, it is a non-biodegradable compound and better solutions must be found. The wetting agent is used to emulsify the unsaponifiable materials and the nonionic surfactants are preferred because of their high emulsification capacity (Dochia, 2013). Beside enzymes and auxiliary substances, in the bioscouring treatment, the results presented in the literature showed the efficiency of ultrasound energy. A frequency between 40-270 kHz does not affect the activity of the enzymes and reduces the treatment time (Easson, 2018). The study of a new eco-friendly method for the pretreatment of textile fabrics is based on the future trend to protect the environment by using natural, biodegradable, and non-aggressive chemicals for the industrial procedures (Shanmugavel, 2018). The aim of the current study was centred on the evaluation of the experimental results obtained for a new eco-friendly method based on enzymatic scouring of cotton/hemp blended fabric where a biodegradable complexing agent (sodium citrate), a commercial enzyme (mixture of pectinases) and ultrasound energy were used. The new approaches for textiles pretreatment: enzymes, biodegradable auxiliaries and ultrasound, could successfully represent an eco-alternative to the aggressive alkaline treatment, by decreasing wastewater pollution, treatment time and temperature with minimal impact on material properties (Erdem, 2018).

Material and Methods

Samples of 30% of hemp+70% of cotton woven fabric with 120±3 cm width and 220±10 g/m^2 weight were used for the scouring treatments. The treatments were done in water with the addition of a commercial enzymatic product Beisol PRO (a mixture of pectinases from CHT-Group Company), a biodegradable complexing agent (2 g/L sodium citrate from Sigma-Aldrich) and a surfactant (0.5% Denimcol Wash RGN from CHT-Group Company). The liquid to fabric ratio was 20:1 and the temperature was kept at 55^{0}C. Different enzyme concentrations between 1-3% o.w.f were used at different treatment time (between 15-55 minutes). To facilitate the access of the enzyme to the substrate, the ultrasound energy at a frequency of 45 kHz was used in ultrasonic bath Elmasonic X-tra basic 2500 from Elma Company, Germany. After enzymatic treatments, a warm wash (70°C) of the treated samples was performed, followed by a cold wash and drying at ambient temperature. For a better evaluation and comparison, a conventional alkaline scouring was done with 10 g/L sodium hydroxide, 5 g/L sodium carbonate, 1 g/L sodium bisulfite, 2 g/L sodium silicate (from Sigma-Aldrich) and 2 g/L Sulfolen 148 (S-148, alkyl polyglicol ether- CHT-Group Company) as a wetting agent. The alkaline scouring was performed at 100^{0}C for 1 hour. The chosen conditions for the scouring treatments of hemp-cotton fabrics are presented in Table 1.
Before scoring, for all samples a preliminary wash was done at 100°C with an AATCC standardized Lander-Ömeter, model M228-AA from SDL Atlas Company-USA, followed by: drying, conditioning and weighing.

Table 1. The chosen conditions for scouring treatments of hemp-cotton fabrics

Sample	Enzyme [% o.w.f]	Time [min]
S1	1.30	21.00
S2	2.70	21.00
S3	1.30	49.00
S4	2.70	49.00
S5	1.00	35.00
S6	3.00	35.00
S7	2.00	15.00
S8	2.00	55.00
S9	2.00	35.00
S10	2.00	35.00
S11	2.00	35.00
S12	2.00	35.00
S13	2.00	35.00
AS	Alkaline sample	60.00
BS	Blank sample	-

In order to determine the new properties of the enzymatic and alkaline scoured fabrics, the weight loss, hydrophilicity, tensile strength, elongation at break, whiteness and yellowness index and CIELAB colour system analysis of the dyed samples with alizarin and ruthenium red dyes were performed. Before testing, the treated samples were conditioned in a standard atmosphere with relative humidity of 65 ± 2% at 21 ± 1°C.
The gravimetric method was used to determine the weight loss. The treated samples were dried at 105°C in an oven from Caloris Group, Romania till constant weight and after that the weight loss was calculated by following equation:

$$\% \text{ weight loss} = (W1-W2)\ x100/W1 \quad \textbf{(1)}$$

where, W1 and W2 are the weights of dried samples fabric before and after treatments, [g].

AATTCC Test Method 79-2007 was used for the hydrophilicity testing. Because during the treatment, fibres degradation may appear by oxidation or hydrolysis reactions, the tensile strength and elongation at break of the fabrics were determined with a testing machine 5KT from Tinius Olsen-United States connected to PC by Horizon software. The ASTM D 5035-06 "*Standard Test Method for Breaking Force and Elongation of Textile Fabrics (Strip Method)*" was used. Datacolor 500 spectrophotometer was used to measure whiteness and yellowness index of the scoured fabrics. Each sample was folded in four plies for an opaque view, and the measurements were made in four different points. The Whiteness and Yellowness Index (CIE/E313) were automatically calculated by Datacolor Tools 2.0 software. Dyeing with ruthenium red and alizarin was performed to determine the remnant pectin from the fabrics after all type of treatments. For this, the samples were measured with Datacolor 500 spectrophotometer (the reflectance-R% at 540 nm) and the colour strength K/S values were calculated. All analyses were made in triplicate. The results presented are the average of the values obtained in each case.

Results and Discussion

The results obtained for hydrophilicity and weight loss of the treated samples are presented in Table 2.

Table 2. Hydrophilicity and weight loss of the samples subjected to the alkaline and bioscouring treatments in different conditions

Sample	Hydrophilicity [seconds]	Weight loss [%]
S1	0.98	1.02
S2	0.88	1.12
S3	0.85	1.24
S4	0.74	1.35
S5	0.86	0.96
S6	0.74	1.60
S7	1.19	0.86
S8	0.76	1.30
S9	0.79	1.39
S10	0.79	1.37
S11	0.73	1.36
S12	0.77	1.38
S13	0.73	1.32
AS	0.45	5.71
BS	Does not absorb	-

All hydrophilicity values are below one second except for the S7 sample (2% o.w.f. enzyme and 15 minutes treatment time). A hydrophilicity lower than 10 seconds is considered good and below 1 second, very good. The values of the enzymatic treated samples are close to the value of the alkaline one, which shows the effectiveness of treatments in removing non-cellulosic attendants. The values for the weight loss are in agreement with data from the literature (Bashar, 2015) for all treatments. Values between 5-10% are reported for alkaline scouring and below 5% for bioscouring (enzymatic treatments).

In order to evaluate the influence of the applied treatments on the mechanical properties of the fabric, the tensile strength and elongation at break were determined. The obtained values of enzymatic and alkaline treated samples are presented in Figure 1.

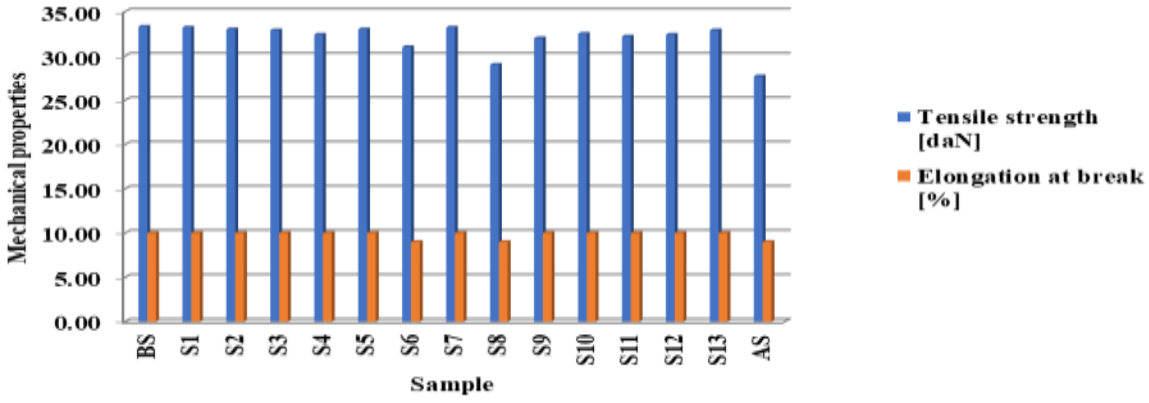


Figure 1. Tensile strength and elongation at break of treated samples

For the most bioscoured samples the tensile strength was not affected, only for S6 (3% enzyme and 35 minutes) and S8 (2% enzyme and 55 minutes) a decrease of 6.91% and 12.91% was observed. The highest decrease in tensile strength (16.82%) was presented by the alkaline treated sample. As for the elongation at break, there were no major changes for any of the samples.

After all type of treatments, an improvement of the whiteness degree was observed both for the enzymatic and alkaline treated samples (Figure 2).

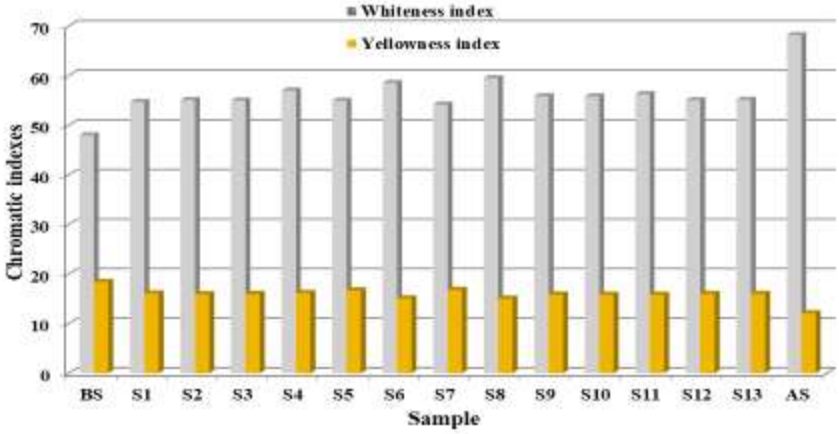


Figure 2. The Whiteness and Yellowness index for enzymatic and alkaline treated samples

The reflectance (R%) at 420 nm was measured in comparison with a standard from barium sulphate of absolute white and the Whiteness Index and Yellowness Index (CIE/E313) were calculated. From figure 2, it can be observed that the conventionally alkaline treated samples present the higher Whiteness Index, which is normal considering that high concentrations of alkali were used. The enzymatic treatments led to a lower Yellowness Index with the enhancement of whiteness degree of the samples compared to blank sample. A higher increase of Whiteness Index is presented by the samples S6 (3% enzyme and 35 minutes) and S8 (2% enzyme and 55 minutes) where a higher enzyme concentration or a longer treatment time were used. In correlation with the increase in the whiteness degree, the yellowness index decreased for all treated samples.

The efficiency of the applied treatments was also highlighted by the identification of the remnant pectin from the treated material. Spectrophotometric analyses in CIELAB system were used for measuring the reflectance (R %) at 540 nm of the samples dyed with alizarin and ruthenium red. The colour strength values of dyed samples are presented in Figure 3.

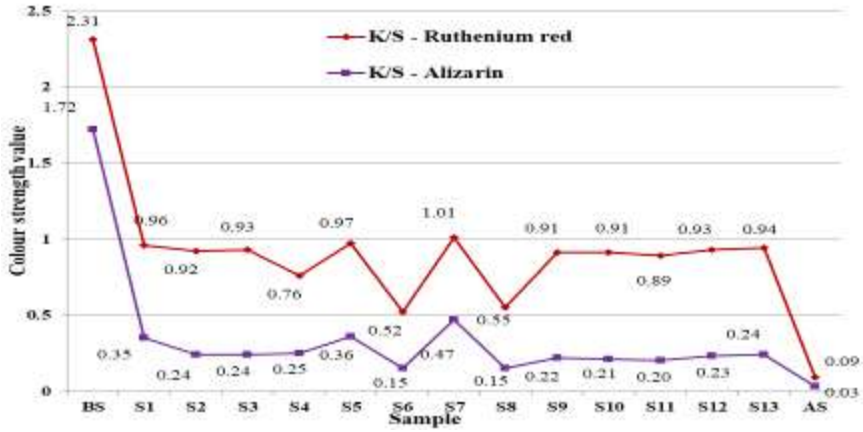


Figure 3. The colour strength [K/S] of the samples dyed with ruthenium red and alizarin

From the K/S values it can be observed that of the enzymatic treated samples, the lowest amount of residual pectin is shown by the samples S6 (3% enzyme and 35 minutes) and S8 (2% enzyme and 55 minutes). For alkaline treatment, K/S value is almost 0. This demonstrates the removal of pectin and calcium ions from the material.

Conclusions

For the studied eco-friendly scouring method, a commercial enzyme product was used in combination with a biodegradable complexing agent (sodium citrate) and ultrasound energy. Thus, it has been proven that by using biodegradable products and lower temperatures in the presence of ultrasound, comparable results with the conventionally alkaline treatment can be obtained. The effect of different treatment conditions was studied by analysing treated samples in terms of weight loss, hydrophilicity, tensile strength, elongation at break, Whiteness and Yellowness index and CIELAB colour system analysis of the dyed samples with alizarin and ruthenium red dyes. The results showed that the eco-friendly method performed in the presence of sodium citrate and ultrasound was efficient without a significant change in the mechanical strength properties of the material at 3% enzyme concentration and 35 minutes treatment time, thereby reducing the costs and the degradation possibility of the treated material.

Acknowledgement

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STUDY OF REPRODUCTIVE INDEX OF WILD BOARS (*SUS SCROFA*) IN CONTROLLED FARMING IN BOSNIA AND HERZEGOVINA

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Abstract

Wild boar (*Sus scrofa*) is an attractive game species that belongs to the genus Sus of the Suidae family of artiodactyl mammals. It is an omnivore that feeds on roots, bulbs, acorns and other fruits, rodents, etc. It is active in the afternoon and at night; it lives in herds that are during the time of mating joined by other old boars (which are solitary).
Wild boar is a widespread species of wild animal. Its natural habitat is in Europe (excluding Scandinavia and the island countries), in Central and South Asia, North and South America, and in Australia. In Bosnia and Herzegovina, wild boar is a widespread game animal and for many reasons is increasingly being reared even in controlled farming.
Training of hunting dogs and selling wild boar meat are common reasons for wild boar farming in our country as well as in the surrounding countries. Reproductive parameters are extremely important from the aspect of wild boar management. A high reproductive capacity of wild boars is based both on early first breeding and on their high productivity. During our research we monitored the situation in the herd that is important for reproduction (the number of units, gender ratio, age, habitat, climate, diet) - we established the number of piglets in litter upon birth as well as the number and gender of live piglets, their body mass upon weaning (day 21).
The studies have shown that there are certain differences in the studied parameters, which is the consequence of different farming methods, climatic factors, habitats, and such studies are extremely important both for the controlled farming of wild boars and for their protection in natural environment, as great hunting potential.

Key words: *wild boar, controlled farming, reproduction*

Introduction

Considering the mountainous configuration of Bosnia and Herzegovina (BiH), widespread vegetation all year long, habitat and controlled farming have made wild boars the most wanted game in our region. This has somewhat contributed to the development of hunting tourism in our region, which still does not occupy the place that it deserves in the overall development of this industry branch in BiH. Boar hunting in the region is mostly done in groups, through "chasing". This type of hunting is an attraction for hunters, however, this type of hunting on the other hand has far-reaching consequences for the breed's foundation stock. In a vast majority of cases this type of hunting does not practice culling. Because of this, the hunting ground owners need to engage in a more intensive and sustainable management of this game that attracts many tourists from the neighboring EU countries. Yet, the wild boar farming is not an easy task for the hunting ground owners. To ensure high-quality offspring and that the reproductive parameters are at a high level, it is necessary to provide a high-quality diet all year long without harming agricultural crops. There is also another set of obstacles that farmers encounter, such as climate changes, expansion of the wild-boar area to abandoned estates, "abandoned" villages, thus causing serious damage to the shredded agricultural parcels that are frequently a source of living for rural population.

Material and methods

The study was carried at three sites in Bosnia and Herzegovina. Two breeding sites were in Kreševo (breeding sites A and B) and one breeding site was in Bratunac (breeding site C). The breeding sites A and B were located at 43°52′N, 18°03′E, while the breeding site C was at 44°12′N, 19°19′E. All three sites were organized as fenced sections within natural habitat consisting of deciduous forests of oak, beech, and hornbeam treas. The fenced section provided a place for bilge, i.e., natural streams. In addition to food found in nature, swine were fed grains as well as swill (scrap from public kitchens i.e., households). The implemented study included three breeding sites that had different numbers of heads for breeding (Table 1).

Table 1. Ratio by gender and age of breeding females and males within a breeding site

	Number and age of breeding females			
	1-2 years	3-4 years	5-7 years	Males
Breeding site A	3	7	1	3
Breeding site B	1	3	2	1
Breeding site C	6	4	0	2

All three breeding sites that were the subjects of our interest had a total of 27 breeding females, which we divided by age in three categories (1 to 2 years, 3 to 4 years, 5 to 7 years). The A herd comprised of 11 breeding females and 3 males. Three females were 1 to 2 years of age; 7 females were 3 to 4 years of age, while 1 female was 5 to7 years of age. The B herd had a total of 7 breeding females and 1 male. One female was 1 to 2 years of age, while 3 females belonged to the category of 3 to 4 years of age. This breeding site also had 2 females that were 3 to 4 years of age and 2 females between 5 and 7 years of age. The C site foundation stock comprised of 12 heads. Ten of them were the breeding females that belonged to two age groups. Six females were 1 to 2 years of age, while 4 females where 3 to 4 years of age. This breeding site also had 2 males. Not a single head from the foundation stock that was the subject of our observation showed any clinical signs of disease. Under the observation period, the heads were subject to antiparasitic treatment. The situation within the herd was monitored through direct and regular presence at the breeding sites, looking out to collect and record information on reproduction. The study examined the mode of management, number of units capable of breeding, ratio between gender and age of the breeding heads. In addition, the study analyzed the habitat in which the animals were reared, and we have established that the habitat together with climate and farm type fit into the natural environment in which wild boars usually live. Counting piglets in litter and recording them by gender was carried out maximum 24 hours after weaning. After day 21, we recorded the number of live piglets, their gender and body weight (kg). The statistical processing of data was implemented using the SSP program for statistical processing of data.

Results and discussion

The mating of wild boar depends largely on dietary conditions, diet, forest fruit yield. 85% of all sows are impregnated in November, December, and January. The mating peak is in mid-December when two-year old sows mate. Pregnancy usually lasts between 112 and 120 days. Sows that are above 3 years of age farrow between six and eight piglets whereas a two-year old sow farrows between three and six piglets. The population of wild boar has grown in the countries in the region in recent years and the measures have been undertaken to assess the reproductive capacities of this species (Šperm, 2012). On the other hand, the studies have

shown that age is very important in reproductive activity, and under favorable living conditions and with adequate diet wild boars are capable of fertilization at a little over 8 months of age (Schlichting, 2015). The dynamics of wild boar farrowing on Medvednica in the Republic of Croatia is similar to that in Austria (Martys, 1982) because the culmination lasts almost 7 months (January-July). Because the period from March to July stands out in the dynamics, it shows similarity to the dynamics of farrowing in Germany (Briedermann, 1971). In our study, which monitors the situation in the herd that is important for reproduction (number of heads, gender ration, age, habitat), we have determined the number of piglets in litter upon birth as well as the number and gender of live piglets and their body weight at weaning (day 21). By comparing the results, the breeding sites A, B and C, we can state that the total number of male livebirths (n=62) was higher in relation to the number of female piglets (n=45), which represents a significant difference in gender ratio, $p<0,05$ (Table 2).

Table 2. Overall study results; number of females; total number of piglets; number of male of female piglets

N	TOTAL	Xi-X	(Xi-X)2	M	Xi-X	(Xi-X)2	F	Xi-X	(Xi-X)2
1	**4**	**1.10**	**1.21**	3	-0.05	0.00	1	1.14	1.30
2	**4**	**1.10**	**1.21**	2	0.95	0.90	2	0.14	0.02
3	**7**	**1.90**	**3.61**	4	-1.05	1.10	3	-0.86	0.74
4	**5**	**0.10**	**0.01**	2	0.95	0.90	3	-0.86	0.74
5	**4**	**1.10**	**1.21**	2	0.95	0.90	2	0.14	0.02
6	**6**	**-0.90**	**0.81**	4	-1.05	1.10	2	0.14	0.02
7	**3**	**2.10**	**4.41**	1	1.95	3.80	2	0.14	0.02
8	**5**	**0.10**	**0.01**	2	0.95	0.90	3	-0.86	0.74
9	**7**	**-1.90**	**3.61**	5	-2.05	4.20	2	0.14	0.02
10	**9**	**-3.90**	**15.21**	5	-2.05	4.20	4	-1.86	3.46
11	**4**	**1.10**	**1.21**	4	-1.05	1.10	0	2.14	4.58
12	**3**	**2.10**	**4.41**	1	1.95	3.80	2	0.14	0.02
13	**5**	**0.10**	**0.01**	4	-1.05	1.10	1	1.14	1.30
14	**7**	**-1.90**	**3.61**	3	-0.05	0.00	4	-1.86	3.46
15	**4**	**1.10**	**1.21**	2	0.95	0.90	2	0.14	0.02
16	**6**	**-0.90**	**0.81**	3	-0.05	0.00	3	-0.86	0.74
17	**3**	**2.10**	**4.41**	3	-0.05	0.00	0	2.14	4.58
18	**5**	**0.10**	**0.01**	4	-1.05	1.10	1	1.14	1.30
19	**5**	**0.10**	**0.01**	2	0.95	0.90	3	-0.86	0.74
20	**6**	**-0.90**	**0.81**	3	-0.05	0.00	3	-0.86	0.74
21	**5**	**0.10**	**0.01**	3	-0.05	0.00	2	0.14	0.02
	107		**47.81**	62		26.95	45		24.57

The total number of live births on day 1 was 107 (males and females), and on day 21, at weaning it was 74, which shows a difference of 30.84% of loss of live-born piglets compared to day 1. Looking at the mean values of the surviving units within the studied groups, it was 5.09 on day 1 while on day 21 it was 3.52, which is a significant difference, the ratio of the

new-born to surviving piglets after weaning from mother. By comparing the number of surviving piglets by gender on days 1 and 21, we have established that the number of surviving male piglets is higher than the number of female piglets. The mean value of surviving male piglets on day 21 at weaning was 2.09 (on day 1 it was 2.95), while the mean value of surviving female piglets on day 21 was 1.42 (on day 1 it was 2.14), which, when comparing the gender ratio of surviving piglets until weaning, on day 21 of life, is a significant difference in favor of the male units that, expressed in percentages, is 29.03% total loss of male piglets and 33.33% loss of female piglets. Body weight was compared on day 21 of life. The mean value of the body weight in males was 2.76 kg, while it was 2.28 kg in females, which is a significant difference in body weight between genders, $p< 0,05$, a high significant difference. In the coastal wetlands area, supply of food throughout the year and high consumption of crops (corn, sunflowers) are probably the explanation for a high reproductive performance in the observed period. In addition, there is also a positive correlation between the size of the litter and the geographic latitude in the European countries, except in Central Europe (Rosell et. al., 2012)

Conclusions

The wild-boar farming and monitoring are demanding and not easy either for farmers or hunters. Active monitoring and analysis of reproductive performances are imperative in promoting and protecting the species, with a close attention to culling in order to improve and protect the population. The reproductive parameters in wild boar point to the necessary changes in management of the species. Significance of differences indicates monitoring of female populations in the research area.

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PHYTOBIOTICS – EFFECTS AND SIGNIFICANCE ON ANIMALS

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Abstract

Spices and herbal extracts are one of the oldest additives in the human diet. In recent years, herbal extracts have become increasingly important as additives in animal nutrition. Alternative growth stimulants, probiotics, prebiotics, organic acids, enzymes, as well as herbal additives with a certain stimulant effect and antimicrobial effect (phytobiotics) have increasingly been used in animals due to the ban on using antibiotics in animals. Phytobiotics are supplements of plant origin in animal feed and affect the stimulation of food consumption, act anti-inflammatory and immunostimulatory, and have the antimicrobial, antioxidant and anticancer effects. Essential oils are synthesized in most plant organs, deposited in secretory cells, cavities, the epidermis, or glandular hairs. They are produced and secreted from glandular trichomes, which are specialized secretory tissues on the surface of plant organs, especially flowers and leaves. They have an antibacterial effect due to their hydrophobicity, which helps them bind to the phospholipid layer of a cell membrane or inhibit bacterial enzymes. Essential oils also have effects on animals in confined spaces that are either calming or stimulating and show reductions in stressogenic effects. The objective of this paper is to point out these and other positive effects of the use of phytobiotics on different species of animals. Further research and studies are necessary to clarify the various nutritional aspects of phytobiotics because, even if additives are considered to be natural products, they should still be tested for possible adverse effects on animal health and for possible interactions with other nutrients.

Keywords: *Phytobiotics, probiotic, food, essential oils, animals*

Introduction

Plants and animals are closely connected. Animal droppings are used in the form of manure and liquid manure to enrich the soil, indirectly affecting plant growth and development (Grčak et al., 2019). On the other hand, products of plant origin, such as phytobiotics, can have multiple positive effects on animals. This can be considered as a form of phytotherapy. The application of phytotherapy has been increasingly intensive since organic livestock production began its development (Grčak et al., 2018). Recent clinical trials have highlighted the specific positive effects of traditional Iranian essential oils on human physiological and psychological parameters (Sharifi-Rad et al., 2017).

According to the Rulebook on Animal Feed Quality (Official Gazette of the Republic of Serbia number 41/09, Article 88, 2010), phytobiotics are classified as growth promoters. Phytobiotics are essential oils (EO) or aromatic plant essences with antimicrobial properties and are classified as sensory additives (European Parliament and Council (2003)). The U.S. Food and Drug Administration (FDA, 2019) has confirmed that essential oils extracted from herbs and spices are safe for use in animals. The resistance of various parasites to antiparasitic drugs is common; the use of drugs implies prolonged toxicity, a long withdrawal period,

which is an important problem for animal breeders. These are some of the most important reasons that have led researchers to turn to alternative remedies based on plants.
The aim of this review paper was to present different positive effects of phytobiotics on different species of animals.

Phytobiotics - properties and significance

Plants produce essential oils as an organic product of their secondary metabolism (Gheisar and Kim, 2017). Essential oils (or their active substances) are safe to use and are a potentially attractive alternative to current antiparasitic drugs (Baltić, 2019). Herbal essential oils have insecticidal and nematocidal effects against a number of insects and nematodes. Thymol as an active component of thyme (*Thymus serpyllum*) was used in the treatment of ascariasis and ankylostomyosis in humans. Special attention is paid to herbal food against endoparasite infections, which can replace conventional therapies with anthelmintics. This special food may contain herbal essential oils or their active ingredients (Marjanović, 2019). Phytogenic additives in animal feed (phytobiotics) are compounds of plant origin and are used to improve animal production performance,the quality of animal origin food and many other effects (Figure 1). They stimulate animal growth by utilizing their physiological potential (Radulović et al. 2015).
They have an antibacterial effect due to their hydrophobicity, which helps them bind to the phospholipid layer of a cell membrane or inhibit bacterial enzymes. Phytobiotics or herbal extracts also have the following effects: anti-helminthic; coccidiostatic; antiviral, antiparasitic and antioxidant. Phytobiotics - herbal extracts, with their specific aroma, influence the secretion of digestive juices and the intestinal motility, and stimulate the endocrine system (Šefer et al., 2015; Nedeljković-Trailović, 2018).

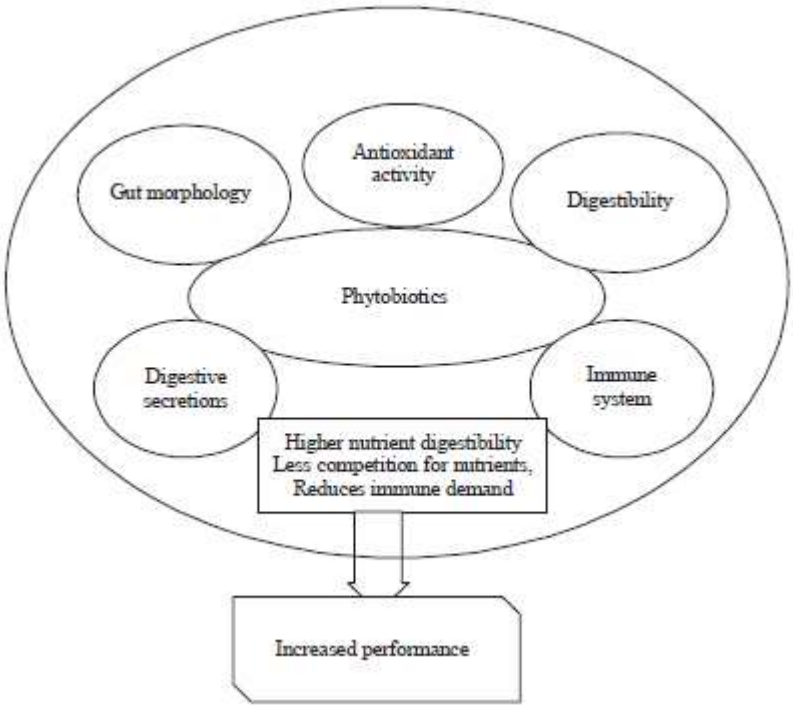


Figure 1. Scheme of activities of phytobiotics in animals (Prabakar et al., 2016)

The use of phytobiotics in sows has proven useful. It has led to an increase in feed consumption during lactation and assured bowel health by preventing oxidative stress and reducing inflammatory processes (Soto, 2013). The use of phytobiotics as growth promoters in the stimulation of weaned pigs (raising pigs) has shown its justified use by achieving better

production results than the control group (without phytobiotics). The experimental group achieved a higher body mass (27.19 ± 4.77 kg), a higher average daily weight gain (0.46 ± 0.09 kg) and better feed conversion (2,043) (Radulović et al., 2015; Gregačević et al., 2014).
Phytobiotics do not create residues (like antibiotics), are naturally derived from plants, and are poorly toxic. The main components of phytobiotics are essential oils, which contain aldehydes or phenols (cinnamaldehyde, citral, carvacrol, thymol, eugenol). Phytobiotics are divided into four subgroups on the basis of their origin and the method of production: herbs, spices, essential oils, and oily resins (Nedeljković-Trailović, 2018; Radulović, 2014).
One of the possible alternatives to synthetic drugs is the use of essential oils (EO), which, to a greater or lesser extent, have one or more pharmacological effects, of which the antibacterial effect is particularly important for veterinary medicine. The antimicrobial activity of carvacrol and thymol against *Salmonella enteritidis* in vitro was researched both individually and in combination, as well as the possibility of their therapeutic use in broilers artificially infected with *Salmonella enteritidis*. The percentage of cured animals treated with carvacrol and thymol was 72.22%, which was 5% less than the control group treated with amoxicillin (77.78%); there was no synergism between carvacrol and thymol (Ratajac et al., 2008).
The application of a mixture of essential oils, consisting of thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*) and rosemary (*Rosmarinus officinalis*) had an effect on production properties and the quality of broiler chicken meat. They showed positive effects on production properties, the biochemical parameters of blood, the histo-morphological parameters of liver and intestinal fibrils, better digestibility of nutrients, the quality of the upper body and meat of chickens, as well as the economy of production itself. (Popović, 2019)
Spices and herbal extracts are one of the oldest additives in the human diet. In recent years, herbal extracts have become increasingly important as additives in animal nutrition. The dominant substances (about 85%) in essential oils determine the antibacterial/biological properties of the oils (Bakkali et al., 2008).
Plants have a primary metabolism, during which changes in sugar molecules, amino acids or fatty acids occur. This primary metabolism is supplemented by the secondary metabolism (which is the adaptation of a plant species to external conditions), whereby compounds called secondary metabolites are formed. The secondary metabolism in plants occurs mainly in specific cells and is not necessary for the function of those cells, but benefits the plant as a whole. Secondary metabolites prevent the attacks of bacteria, fungi or viruses (phytoalexin function) on plant tissues. They also protect the plant from excessive ultraviolet radiation, excessive transpiration, etc. The major secondary metabolites are alkaloids, heteroids, saponosides, tannins and terpenoids. Terpenoids are the basis of essential oils. (Radulović, 2014)
Despite major chemical differences, the main components of common essential oils can be classified into two structural families: terpenoids and phenylpropanoids. Both of these groups contain phenolic compounds. Phenolic compounds act as antioxidants due to their reactivity with peroxyl radicals. Due to its stability, a phenoxyl radical will not create new chains of radicals, but will wait for a new peroxyl radical and then, in a radical-radical reaction, these radicals will nullify each other. Chain-breaking antioxidants significantly inhibit auto-oxidation at low concentrations ($<10^{-3}$), even in the presence of radical initiators, until they are consumed. The antioxidant activity of essential oils is also conditioned by the climatic factor that affects the composition of these oils. The oil extraction technique also affects the oil quality. For example, when supercritical fluid extraction (SFE) was used, the eugenol content in *Pimenta dioica* was 77.4%, and when water distillation was used, this percentage was 45.4% (Morongiu et al., 2005; Dimić et al. 2017). There are several techniques for extracting essential oils from different parts of plants. The most used techniques are water distillation or

steam distillation, pressurized-solvent extraction and subcritical water extraction (Radulović, 2014).

The effect of two different constituents of a mixture of essential oils on the proliferation of *Clostridium perfringens* in the intestines of egg-laying hens (broilers) showed that the results were similar to those that used antibiotics (Mitsch et al., 2004). Based on the data (Šević, 2016) on the effect of broiler diets with the addition of various phytogens (thymol + cinamaldehyde; essential oils of caraway, peppermint, clove and anise; and thymol), some conclusions can be drawn. The experimental groups (with phytogens) had a better health status, yield parameters, production results, meat quality, and gut microbial status. The control group (phytogen-free) had the highest feed consumption and, in the studied parts of the digestive tract of the control group, there was a higher number of *E. coli* and *Enteroccocus spp*. bacteria, and a smaller number of lactic acid bacteria. Phytobiotics and their extracts have a wide range of effects: they stimulate feed consumption and endogenous enzyme secretion, have antimicrobial and coccidiostatic actions, and improve production results, poultry health and product quality. Medicinal plants that can be used as additives include: chamomile, lemon balm, peppermint, fennel, yarrow, thyme, basil, etc. (Pavlovski et al., 2009; Valenzuela-Grijalva et al., 2017). An addition of a mixture of plants in animal feed shows better results due to the cumulative effect that individual plant species have when given in combination (Petkova, 2009). A steady increase in the organic product demand worldwide indicates that this production may be very profitable if natural resources, knowledge and production experience are used properly (Gulan 2018). Research has also been conducted on the possible antitumor and antimicrobial effects of mahogany bark extracts (*Mahonia aquifolium* (Pursh) Nutt.). Testing the cytotoxicity of aqueous and ethanol extracts on human malignant cell lines showed that the extracts had a selective cytotoxic activity, and the highest intensity of action was shown on HeLa[1] cells of cervical adenocarcinoma (Damjanović, 2018). In 2017, Salaheen and others found that supplementation with bioactive phenolic extracts (BPE) in water led to an increase in average chicken body weight by 5.8% and caused a similar effect to antibiotic growth promoters (AGP) on bacterial colonies. There was an increase in Firmicutes and a simultaneous decrease in Bacteroides in chicken feces. These results have shown that the use of bioactive phenolic substances from berries, as an alternative to antibiotic growth promoters, has a potential role in reducing the development of antibiotic resistance among bacterial pathogens. In his paper, Vučinić (2008) presented literature data relating to aromatology as a measure in preventing the onset of disorders in dog behavior. Aromatherapy and aromatology are the branches of phytotherapy, which are based solely on the use of essential oils obtained by distillation and lemon oils obtained by cold straining. Aromatherapy is a term for therapy whose main factor is the olfactory system. Olfactory stimulation with essential oils is considered to be a useful way of enriching housing conditions for many animals kept in confined spaces. Essential oils have effects on animals in confined spaces that are either calming or stimulating and show reductions in stressogenic effects. The effects of four essential oils (lavender oil, chamomile oil, rosemary oil and peppermint oil) on the behavior of dogs in a shelter were tested. Dogs exposed to the scent of lavender oil and chamomile oil spent much less time barking and moving. Peppermint oil and rosemary oil had the opposite effect. In addition, a study conducted by Vučinić in 2008 refers to the calming effect of lavender oil on dogs that get disturbed during transport. Dogs that inhaled the scent of lavender oil during transport spent more time in a recumbent or sitting position, moved less and barked less. Aromatherapy in the form of lavender scents reduced anxiety in dogs during transport. An alternative to synthetic drugs is the use of essential oils, which have one or more pharmacological effects, of which antibacterial activity is particularly important for veterinary

[1] HeLa cell is an immortalised cell line used in scientific research.

medicine. The data on the chemical properties of essential oils are known for only about 500 species of the 1500 aromatic herbs that serve as a source of raw materials for perfumeries. Of these, about 50 species find use as a source of essential oils and aromas (Joy, 1998). The most common natural phytogenic additives (antioxidants) are spices (Mašin (2009); Joy, 1998).
Rosemary (*Rosmarinus officinalis*, L.); Oregano (*Origanum vulgare*); Thyme (*Thymus serpyllum*); Caraway (*Carum Carvi* L.); Frangula (*Rhamnus frangula* L.); Peppermint (*Mentha x piperita* L.); Parsley (*Petroselinum crispum*, Mill); Pomegranate (*Punica granatum*); European Spruce (*Picea abies*); Japanese Umbrella Pine (*Sciadopitys verticillata*); Piper (*Piper acutifolium*); Horseradish (*Armoracia rusticana* or *Cochlearia armoracia*); Meadowsweet (*Filipendula ulmaria*); Coriander (*Coriandrum sativum*); Cumin (*Cuminum cyminum*); Italian Honeysuckle (*Lonicera caprifolium*)…

Conclusion

Animal feed should include several objectives: animal health; good reproductive and production performance; it should not affect the quality of animal products and should not pollute the environment. Therefore, the use of substances of natural origin (probiotics, prebiotics, phytobiotics, etc.) has been imposed increasingly. The justification for the use of phytogenic additives (phytobiotics) in animals stems from their positive effect on animal health, and the qualitative and quantitative increase in production. Phytobiotics can be used as whole plants, parts of plants or essential oils. The ways in which phytobiotics affect animal health are: they increase feed consumption and digestibility of nutrients, improve the appetite; have the antimicrobial activity, inhibit the growth of microorganisms; have the anti-inflammatory effect, prevent the metabolism of inflammatory prostaglandins; the immunostimulatory action, stimulate the function of lymphocytes, macrophages and natural killer cells, promote phagocytosis; antioxidant activity, neutralize free radicals or antioxidant enzymes; have an impact on parasites, the coccidiostatic effect. Phytobiotics do not have residual effects, do not disturb the ecological balance, and are natural products. Their disadvantages lie in the difficulty of their classification and standardization due to a physiologically complex system. The composition of plants, phytobiotics, depends on weather conditions, the soil type, climate, harvest, storage… The most common plants - phytobiotics are: The most common natural phytogenic additives - antioxidants – spices: Rosemary (*Rosmarinus officinalis*, L.); Oregano; Thyme; Summer savory; Pomegranate; Chili pepper; caraway; pepper; artichoke, dog rose, nettle, chamomile, dandelion and essential plant oils, etc. Although additives are considered to be natural products, they should still be tested for possible adverse effects on animal health and for possible interactions with other nutrients.

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TOWARD BETTER PREPARADNESS OF MEDITERRANEAN AGRO-HYDROLOGICAL SYSTEMS TO FUTURE CLIMATE CHANGE-INDUCED DROUGHTS, STUDY CASE OF BOUREGREG WATERSHED (MOROCCO)

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Abstract

Globally, the Mediterranean region is considered among the most vulnerable regions to climate change impacts. This situation puts both agricultural and hydrological systems in this region at high risks. In order to improve the preparedness of both agricultural and hydrological systems to future climate change-induced phenomenon, such as drought, predictive analysis of their vulnerability is crucial. In this study, a hybrid modeling approach was built to understand the response of major crops and streamflow in a Mediterranean watershed to 2085-2100' droughts. The study watershed is Bouregreg catchment (9656 km^2) in Morocco. To achieve this objective, the agro-hydrological model SWAT (Soil and Water Assessment Tool), the drought indices calculator DrinC© and the Indicator of Hydrologic Alteration program (IHA) were forced with climate data of two emissions scenarios (RCP4.5 and RCP8.5) from a downscaled Global Circulation Model. Several drought events, with different intensities across sub-basins, have been identified in the 2085-2100 period under both RCPs. Flow alteration has been estimated at the study watershed for the future simulation period. Crops wise, significant decreases of wheat productivity (up to -65%) were simulated during the future extremely dry growing seasons. Drought assessment at local scale needs reliable approaches capturing both local landscape processes (such as the local cropping patterns) and the watershed-scale hydrological responses. The adopted methodology in this study offers a comprehensive framework by taking benefit of the physical distribution feature of SWAT model and the statistical representation of different drought indices to forecast future droughts and their spatial extent within Bouregreg watershed. The adopted approach could be used in other watersheds with similar context and challenges.

Keywords: *Drought impact, agro-hydrological system, Bouregreg watershed, low flow, crop yield, SWAT, SPI*

Introduction

Being located in one of the global hot-spots of climate change, the Mediterranean region is considered as one of the complex study cases when dealing with climate change projections and their impacts on natural ecosystems (Filipe & Lawrence, 2013). Dryness and the frequency of extreme events (Heat waves, droughts, floods, etc.) are supposed to increase in this region (IPCC, 2019). Most of the Mediterranean countries, including Morocco, have had drought episodes during their recent history with many impacts on social, economic, and environmental sides; and most of the climate observations report that the frequency and the intensity of droughts in the Mediterranean region is in an increasing trend (Hoerling et al., 2012). In addition to the direct impact on crops productivity and freshwater availability,

droughts affect the whole agro-hydrological ecosystems with irreversible harms in some situations (Bucak et al., 2017).

The agro-hydrological modeling has emerged as a promising tool to address both agricultural and hydrological issues, in an integrated approach, while studying phenomenon or setting up management strategies (Tejada, 2010). In the other hand, many drought indices, such as Standardized Precipitation Index (SPI) and the Standardized Precipitation Evapotranspiration Index (SPEI), have proved their reliability in capturing drought occurrence and describing their attributes with very low uncertainty (WMO & GWP, 2016). Several studies tried to address drought impacts from agro-hydrological perspective using drought indices and semi-distributed models, such as SWAT model (Dash et al., 2019; Li et al.,2020); But no previous similar study has been undertaken in Morocco.

By combining the agro-hydrologic modeling and a widely used drought index, for the first time in Morocco, we believe that drought impact on the agro-hydrological systems should be assessed comprehensively. The main objective of this paper is to build a hybrid approach to assess the response of agro-hydrological systems in a Mediterranean watershed (Bouregreg, Morocco) to climate change-induced droughts.

Materials & Methods

Study area

Bouregreg watershed (BW), 9656km^2, is a typical Mediterranean watershed located in the North-western of Morocco. The climate is considered as Mediterranean; average mean temperature is 16°C and average annual rainfall is around 460mm (Khomsi, 2014).

Like most of large Mediterranean watersheds, diverse activities are held within BW; forestry and farming activities are the main ones and cover respectively: 24% and 28% of the watershed land uses. As per hydrology, BW's water budget is dominated by surface water component (90%). The stream network is build around four streams (Figure 1): Bouregreg, Grou, Korifla and Machraa streams; the four streams are gauged by respectively: Aguibate Ziar, Ras Fathia, Ain Loudah and Sidi Mohamed Chrif (S.M.Chrif) streamgauges. The annual potential water resources of BW is 720 million m^3 (ABHBC, 2011).



Figure 1. Location, topography, streamflow network of BW

Agro-hydrologic model

For agro-hydrological modeling, authors used Soil and Water Assessment Tool (SWAT) model (Arnold et al., 2011); which has been successfully used in many eco-hydrological modeling and climate change studies in the Mediterranean area (Brouziyne et al., 2017). SWAT is a semi-distributed model, which divides the study watershed into smaller units, namely Hydrologic Response Units (HRUs), with common land use, soil and elevation classes (Arnold et al., 2012). SWAT is then simulating the hydrologic and plants growth processes in each HRU within the watershed.

For more confidence in SWAT model performance, calibration and validation processes are required. In this study, we used SWAT-CUP (SWAT Calibration and Uncertainty Program) (Abbaspour et al., 2007) to calibrate both hydrologic and plant growth modules of SWAT over BW. To achieve this objective, simulated monthly streamflow (at the four streamgauges)

and simulated annual wheat yields were confronted to, respectively, observed monthly (1990-2005) streamflows at the four streamgauges and the recorded (1990-2005) average wheat yields in the study watershed. During streamflow calibration and validation, the determination coefficient (R^2) and Nash-Sutcliffe coefficient (NSE) were adopted as goodness-of-fit indicators; while for plant growth module calibration and validation, we adopted only the R^2.

Drought Index

The SPI is considered as the most used meteorological drought index where the precipitation data is fitted to gamma distribution (Kang & Sridhar, 2017). More details about the SPI calculation is described by McKee et al.(1993).SPI can be calculated at various timescales: 3, 6, 9, and 12 months (WMO & GWP, 2016); For this study, and since the hydrological systems is the one of focuses, the SPI-12 was calculated for BW using monthly precipitation.

Hydrologic alteration simulator

Indicators of Hydrologic Alteration (IHA) program (Richter et al., 1996) was used to estimate the potential alteration of hydrology in BW under projected droughts. Being developed by The Nature Conservancy to perform hydrologic analysis from ecological perspective, IHA program is based on the relevance of a set of flow attributes to ecological integrity of riverine ecosystems (Richter et al., 1998).

Datasets

Different datasets were collected to build SWAT model for BW conditions, to calibrate its hydrologic and plant growth modules and to simulate future hydrological and plant growth processes. The table xx summarize the used database in this work.

Table 1. Input data and their resolution

Data	**Resolution / time step**
Digital elevation model	30m
Land use/Land Cover	30m
Soil data	1km
Baseline climate	Daily temperature and rainfall (from 1985 to 2005)
Streamflow data	Monthly (1990 to 2005)
Farming pattern	Planting/harvest dates, details of key land and crops management practices, key physiological features of grown varieties
Projected climate	Daily temperature and rainfall (from 2085 to 2100) from downscaled Global Circulation Model (CNRM-CM5)

Approach

After calibration and validation of SWAT model under baseline (1990-2005), projected climate variables were used to force SWAT model to simulate future hydrologic and wheat crop yields in BW under both RCPs (RCP4.5 and RCP 8.5). The simulated future daily streamflow at the four streamgauges were used to run IHA program in order to compute the expected hydrologic alterations. In the other hand, future precipitation data were used to compute the potential droughts events in the study watershed through DrinC© program.

Results & Discussion

Agro-hydrologic model calibration

Sensitivity analysis of both crops development and hydrologic processes in BW revealed that are sensitive to respectively: HVSTI (Harvest index), WSYF (Lower limit of HVSTI), BLAI (Maximum potential leaf area index), BIO_E (Radiation use efficiency), and EXT_COEF (Light extinction coefficient) for the plan growth module, and SOL_AWC (Available water

capacity of the soil layer), CN2 (Initial SCS runoff curve number for moisture condition II), ESCO (Soil evaporation compensation factor), and GWQMN (Threshold depth of water return in shallow flow to occur) for the hydrologic module. After several SWAT-CUP iterations while adjusting the sensitive parameters, satisfactory agreement was achieved between simulated and observed processes; the table 2 represent the agreement indicators values after calibration and validation efforts.

Table 2. Values of goodness-of-fit indicators during calibration and validation

	Hydrology								Crop
	Aguibate Ziar		Ras Fathia		S.M.Chrif		Ain Loudah		Wheat
	R^2	NSE	R^2	NSE	R^2	NSE	R^2	NSE	R^2
Calibration	0.69	0.7	0.73	0.63	0.77	0.87	0.71	0.69	0.62
Validation	0.72	0.62	0.69	0.51	0.52	0.46	0.67	0.5	0.56

Since all R2 and NSE values are exceeding 0.5, the satisfactory agreement threshold set by Moriasi et al.(2007), we can use SWAT model to assess hydrologic and crops development processes in BW.

Future droughts

According to SPI-12 projection in the 2085-2100 period, BW will experience several drought events with different intensities and durations (Figure 2).

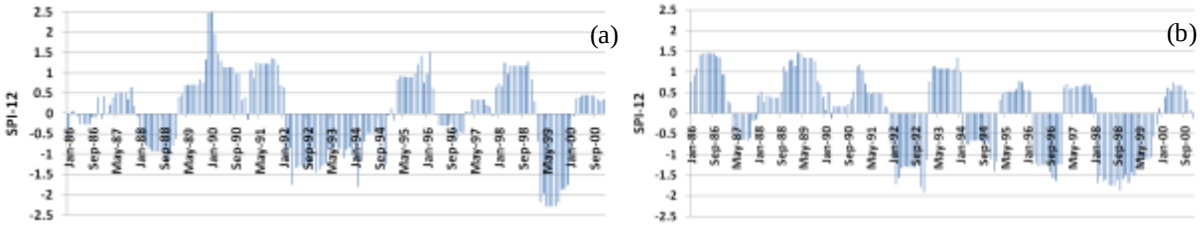


Figure 2. Projected drought event in BW under RCP4.5 (a) and RCP8.5 (b)

Under the emission scenario RCP4.5, simulations show the occurrence of mainly three drought events with the highly extreme drought event predicted in late 2099-early 2100. As per the RCP8.5, more than four drought events are expected; Most of them are either moderate or extreme and distributed along the last 15 years of the simulation.

Flow alteration

To capture the expected flow alteration under future climate change impacts in BW, we focused on monthly low flows as a representative attribute of environmental flow in the Mediterranean context. IHA calculations for BW revealed that there will be a reduction of monthly low flows in comparison to baseline (Figure 3).

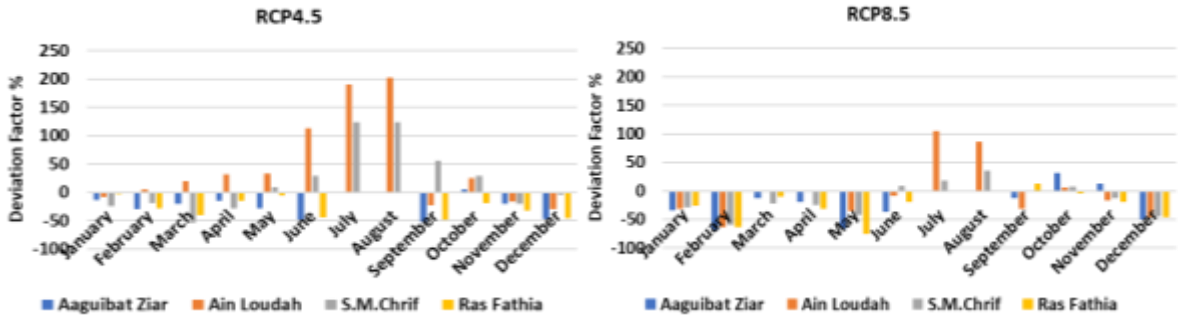


Figure 3. The distribution of deviation factor percentage of monthly low flows

Most of BW's streams will undergo a decrease in low flow means, especially under the pessimistic emission scenario. The computed high positive deviations for some specific

months (July and August) at Loudah and S.M.Chrif gauges can be explained by the very low flows during these months (in baseline) which amplified the deviation even if the change is small.

Such hydrologic modification should have significant impacts on: water table levels in the floodplains, soil moisture distribution (especially in the riparian zone), and dissolved oxygen rates; which might impact farming, water availability and the riverine biodiversity.

Wheat crop performance

Overall, and compared to baseline, annual wheat yield averages should decrease by -14% and -27% under respectively the RCP4.5 and RCP8.5. SWAT's simulations of wheat yield under the projected climate during 2085-2100 period was in agreement with the outcomes of SPI-12 simulations.

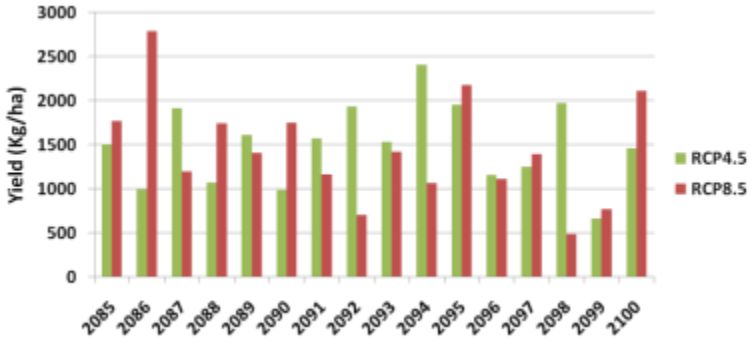


Figure 4. The distribution of projected annual wheat yield under both RCPs

Under RCP4.5, and during the most severe drought period (late 2099 early 2100), very dramatic wheat yield drop was simulated by SWAT (-58% to a normal growing season's yield). Similarly, the most dramatic simulated yield drop under RCP8.5 (-65%) was in 2098 season where the most long drought event is expected.

Conclusion

Bouregreg watershed (BW), like most of the large Mediterranean watersheds, is playing key environmental and socio-economic roles; And should be facing increasing challenges related the planned socio-economic development and the climate change impacts. In order to plan suitable management strategies, it is important to understand the responses of its vital systems (such as: agriculture and hydrology) to future changes; Especially the climate change' related ones. This study revealed that:

- BW will experience many drought episodes with different intensities and durations under the both emission scenarios;
- Monthly low flow, a key component of environmental flow integrity in any river watershed, will decrease in almost all BW streams leading to agricultural, hydrological and ecological impacts;
- Wheat yields will be affected during the dry years and inter-annual variability of yields will keep evolving in the future.

Since the potential extent of future climate change-induced drought has been identified, science-based strategies should be set up to increase the preparedness of the agricultural and the hydrological systems in BW; with the promotion of sustainable solutions and nature-based practices.

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MONITORING NOXIOUS SUBSTANCES FROM COMBINED FEED FACTORIES FOR ENVIRONMENTAL MANAGEMENT

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Abstract

Compound feeds are an important source of food for the animals from zootechnical farms, because in addition to ground cereals they also contain vitamins, minerals, proteins, amino acids, and sometimes even drugs, energizers or flavors. The process of obtaining different varieties of compound feeds involves a complex technological flow, with specific stages of production. Mixing, sterilization and granulation activities, where steam jets are used at very high temperatures determine the elimination of different types of noxious substances in the air. In this article are presented the experimental measurements performed at the critical points of working installations from a compound feed factory, on the entire duration of the assortment lot. Considering the purpose of the performed researches, the critical measurement points were established at the exit of steam generator, where steam jets are obtained at very high temperatures, with values of approximately 150-180ºC. Using a TESTO 350 M/XL gas analyzer, the measured values allow a complete analysis of the types of noxious eliminated. The results of experimental measurements show that the fluctuations obtained during the measurements are very small, of the order 2 mg/m^3_N for each type of noxious eliminated, which indicates a very good regulation of the work process. The average values of the eliminated noxious substances, CO, NO_x, CO_2 indicates compliance with European environmental quality standards. The use of high-performance instalations, with a high degree of automation allows rigorous control over the different types of pollutants released into the atmosphere by the work installations from compound feed factories.

Keywords: *compound feeds, noxious substances, environment, control, automation*

Introduction

Demographic growth and diversification of human needs have led to an upward evolution of activities in the field of livestock. Thus, the use of compound feeds for the feeding of animals on livestock farms has become very necessary, both for the supply of good quality nutrients and for reducing the costs of animal products. Depending on the age of the animals or the species of animals fed, certain compound feed recipes are used, with different compositions, shapes and weights of the granules (Mihaila, 2001; Gaceu, 2006; Şara et al., 2005).

During the technological flow, in a compound feed factory the cereals and mineral substances that make up the desired recipe are mixed, homogenized, granulated and sterilized with the help of high-performance equipment and work facilities. From the combustion equipment which producing the steam necessary for the sterilization and homogenization in various forms of compound feeds and also from the cooling installations of the final product, different types of pollutants are released into the atmosphere (Roden et al., 2006). These pollutants can affect the health of people working in these factories and also the environment.

That is why it is absolutely necessary to monitor and control rigorously the concentrations of noxious substances from the gases emitted by the work installations which have combustion sources or internal combustion engines (Heinsohn and Kabel, 1999; Lailer, 2005; Franke,

2006). At critical working points, that is, at the outlet of the hot steam generator, samples were taken and various experimental measurements were made in order to analyze the functional parameters of the work installations.
The paper presents a complex methodology for measuring the concentrations of different types of pollutants resulting from the process of obtaining compound feeds. Experimental analyzes and measurements for the determination of flue gases emissions from the steam generator outlet were performed using the TESTO 350 M/XL gas analyzer over a period of 100 minutes (which represents the minimum duration for making a batch of compound feed assortment). The experimental studies carried out propose an analysis of the functional parameters of the working installations in the compound feed factory, in order to automate the control of the steam temperature and implicitly of the combustion installation (that determines the elimination of noxious substances in the air). For this purpose, separate locations for experimental measurements were used in the burner area of the steam generator.
Given that each compound feed recipe requires different operating parameters of the work facilities, samples obtained during the technical process for the production of two compound feed recipes were analyzed: for the feeding of broilers, respectively for the feeding of swine.

Materials and Methods

One of the stages of the technological flow from a compound feed factory is that in which the necessary ingredients from the recipe are mixed, which will be performed under the action of steam jets with very high temperatures. These jets are produced by a steam generator and are needed to obtain granules of different shapes and sizes (Bond et al., 2002). Also, the steam jet at very high temperatures has other important purposes in obtaining the final product: homogenization of granules and their sterilization. An essential work equipment from the compound feed factory where the experimental measurements were performed is the steam generator (Certuss Junior), having as primary fuel the liquefied petroleum gas (LPG).
Given the purpose of the research conducted, samples were taken at the outlet of the steam generator and experimental measurements were performed in order to analyze the working parameters of the equipment and installations used (Bollen et al., 2014). Thus, at this critical working point, the pollutant emissions from the flue gases resulting from the process of obtaining the steam jet were measured. During researches on flue gas, the TESTO 350 M/XL gas analyzer was used, in order to obtain the measured values for the noxious substances released at the outlet of the steam generator. This device operates according to the following principle: the change of the current intensity generated by a galvanic cell whose electrolyte changes its properties is analyzed (due to interaction with the gaseous component removed in the air to be detected and whose concentration is to be measured). Thus, the cells used to perform the measurements are in fact galvanic elements, which generate a current proportional to the number of ions that dissociate in the electrolyte solution (as a result of the reaction obtained between the electrolyte and the noxious substance analyzed at that time). Analyzers built according to this principle have a great advantage represented by a low construction cost and are used for short or medium duration measurements. With their help, concentrations can be determined for the following gases: O_2, CO, H_2S, NO, NO_2, SO_2, etc.
The TESTO 350 M/XL gas analyzer is an advanced equipment for determining the noxious substances eliminated by the flue gases, their determination being made in specialized measuring cells (in which Peltier type electrochemical reactions take place). The device used to perform analyzes in the compound feed factory where the research took place, consists of three working components: the analysis unit, the control unit and the gas sampling probe. The effective interaction between the electrolyte and the flue gases takes place in the unit of analysis, because this is where the reaction cells are located (fig. 1). Also here are the supply

batteries of the device, the filters for retaining solid impurities from the flue gases, the condenser decanter and the electro-pneumatic connections with the other components.



Figure 1. The analysis unit: 1 - electrical contacts; 2 - control LEDs; 3 - solid particle filter; 4 - filters for retaining particles from the aspirated air; 5 - condensate collection; 6 - analysis cells; 7 - integrated system for determining the gas velocity and pressure; 8 - connections.

Due to the Peltier type electrochemical reactions that take place inside the analysis unit, an electrical signal is emitted to the control unit of the device, thus displaying the concentration value corresponding to the type of noxious substance analyzed (Ionel, 1994). The TESTO 350 M/XL analyzer has a control unit (fig. 2), which is a device that can be operated with the help of the built-in keyboard, or with a special contact pencil (touch-pen).

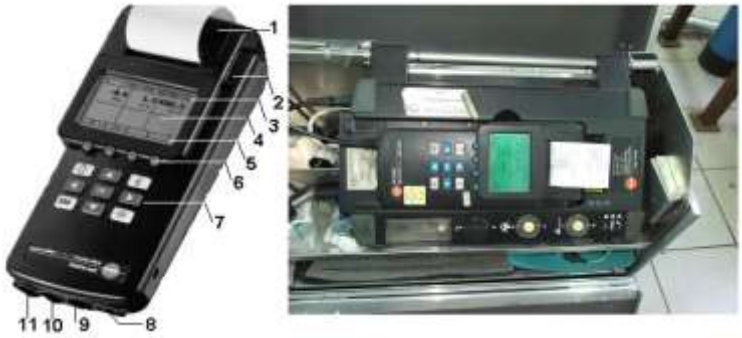


Figure 2. Control unit: 1 - printer; 2 - touch-pen; 3 - system information bar; 4 - display of measured values; 5 - bar for operation information; 6 - function operation keys; 7 - keyboard; 8 - pressure probe connection; 9 - sample connection; 10 - analysis unit connection; 11 - serial interface.

The analyzer can be equipped with several types of gas sampling probes, depending on the characteristics of the sampled gases. To performing the experimental measurements was used a probe with tubing heated at 180°C, operating at temperatures up to 1200°C .
After the three components are interconnected, the analyzer will be switched on by connecting to the 220V mains or using its own batteries. At this point the device enters automatically in the procedure of "zero calibration" and washing of the reaction cells (fig. 3). At the time of "zero calibration", the probe of the device must not be inserted into the flue.



Figure 3. Zero calibration procedure of the Testo 350 M/XL analyzer.

After the analyzer enters in normal operation regimen, it must be programmed to acquire and display the data of interest for the analysis performed (Vasile, 2018). Also now is made the setting for the studied noxious type, from the device database (fig. 4).

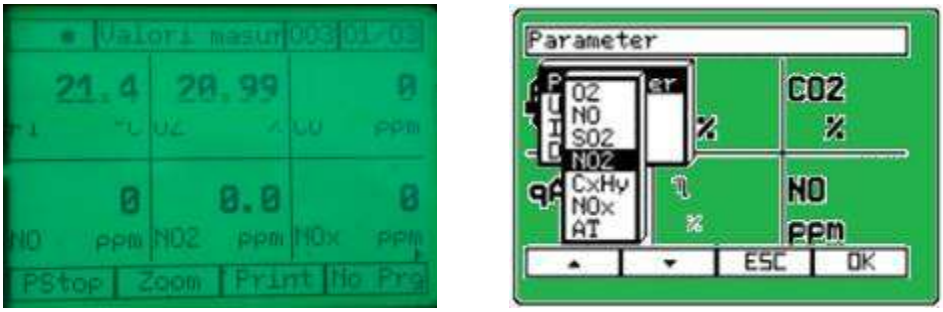


Figure 4. Setting the display of the desired values and the studied noxious type.

After setting the parameters we want to measure, the probe is inserted into the gas channel and the activity of measuring the monitored parameters is started (Vasile, 2018). The values obtained from the analysis and measurements carried out will be compared with the limit values provided in the legislation in force (in order to confirm compliance with the rules imposed by the European Union). The measurements of the noxious concentrations eliminated by the flue gases from the steam generator were performed during the combined fodder production processes for two species of animals, with a large share in the livestock farms: broilers and swine. During 100 minutes (in which an assortment of combined fodder is produced), ten distinct measurements were performed for the noxious substances eliminated in the air (at an interval of 10 minutes between them).
If the TESTO 350 M/XL analyzer will be connected to a computer, it can be used for long term measurements (days, weeks) by running a special program: TESTO Easy Emissions.

Results and Discussion

The thermodynamic parameters of the steam generator in the case of the production of compound feeds for broilers are: steam temperature in the installation 140°C; thermal agent temperature 170°C; nominal steam pressure 6 bar. The results of the measurements for the released emissions are presented in Table 1.

Table 1. Measurement values during compound feed production for broilers

No.	CO [mg/m^3_N]	NOx [mg/m^3_N]	SO2 [mg/m^3_N]	CO2 [g/m^3_N]	CO* [mg/m^3_N]	NOx* [mg/m^3_N]	SO2* [mg/m^3_N]	CO2* [g/m^3_N]
1	46.25	14.37	0.00	182.44	50.79	15.78	0.00	200.36
2	46.25	14.37	0.00	182.44	50.79	15.78	0.00	200.36
3	42.50	10.26	0.00	182.44	46.67	11.27	0.00	200.36
4	45.00	14.37	0.00	182.63	49.69	15.87	0.00	201.68
5	45.00	16.42	0.00	182.63	49.09	17.91	0.00	199.24

6	41.25	14.37	0.00	182.63	45.55	15.87	0.00	201.68
7	45.00	14.37	0.00	182.63	49.69	15.87	0.00	201.68
8	45.00	14.37	0.00	182.44	49.42	15.78	0.00	200.36
9	42.50	14.37	0.00	182.44	46.67	15.78	0.00	200.36
10	45.00	14.37	0.00	182.63	49.69	15.87	0.00	201.68
Average	44.38	14.16	0.00	182.54	48.81	15.58	0.00	200.78

*) values relative to the reference oxygen 3%

The results of the experimental measurements of the noxious concentrations eliminated in the production of compound feed for broilers are presented in graphical form in figure 5.

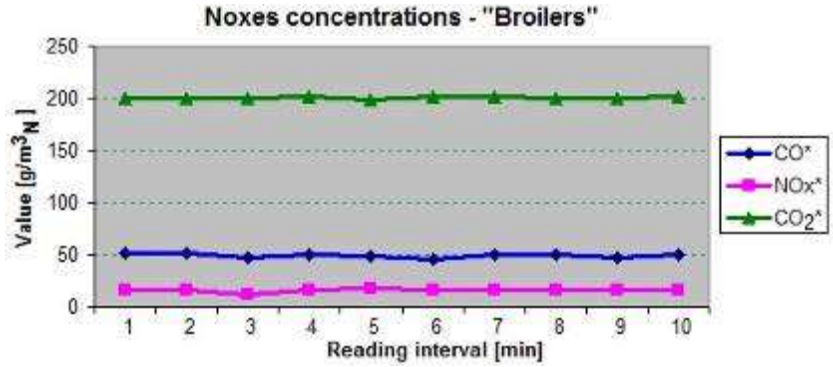


Figure 5. Concentrations of emitted pollutants in the case of compound feed for broilers.

The thermodynamic parameters of the steam generator in the case of the production of compound feed for pigs are: steam temperature in the installation 155°C; thermal agent temperature 180°C; nominal steam pressure 7.5 bar. The results of the measurements for the released emissions are presented in Table 2.

Table 2. Measurement values during compound feed production for swine

No.	CO [mg/m^3_N]	NOx [mg/m^3_N]	SO2 [mg/m^3_N]	CO2 [g/m^3_N]	CO* [mg/m^3_N]	NOx* [mg/m^3_N]	SO2* [mg/m^3_N]	CO2* [g/m^3_N]
1	63.20	17.33	0.00	170.20	75.61	20.34	0.00	203.74
2	63.24	17.35	0.00	170.35	75.61	20.57	0.00	203.96
3	63.25	17.41	0.00	171.08	76.60	20.70	0.00	206.28
4	61.85	19.37	0.00	171.31	75.10	23.56	0.00	208.62
5	61.93	19.24	0.00	172.15	75.25	23.73	0.00	208.40
6	61.95	19.42	0.00	172.40	75.30	23.80	0.00	208.54
7	62.15	19.46	0.00	172.69	75.10	23.17	0.00	208.79
8	61.82	17.30	0.00	172.82	74.18	20.61	0.00	206.85
9	63.20	17.18	0.00	170.95	75.61	20.48	0.00	204.12
10	63.30	17.36	0.00	171.26	76.05	20.55	0.00	205.98
Average	62.59	18.14	0.00	171.52	75.44	21.75	0.00	206.53

*) values relative to the reference oxygen 3%

The results of the experimental measurements of the noxious concentrations eliminated in the production of compound feed for pigs are presented in graphical form in figure 6.

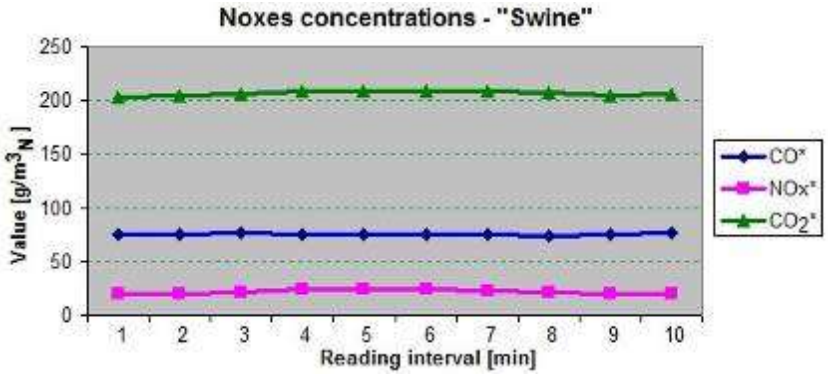


Figure 6. Concentrations of emitted pollutants in the case of compound feed for swine.

The measured values of the noxious substances from the flue gases for the two varieties of feed combined indicates very small fluctuations of the order 2 mg/m^3_N for CO, NO_x, CO_2 and also that SO2 is not eliminated in the air as an exhaust pollutant.

Conclusions

In order to increase the labor productivity in the combined feed factories, the aim is to increase the degree of automation of the activities in the technological flow, so as to ensure a fast and accurate measurement of the working parameters, required in obtaining the desired combined feed recipe. Meeting the requirements for pollution standards accepted in the European Union requires the use of high-performance work facilities, with a high degree of mechanization, automation and computerization.

Analyzing the measured values of the noxious substances from the flue gases for the two varieties of feed combined, it can be observed that the fluctuations obtained during the measurements are very small, of the order of 2 mg/m^3_N, for each type of noxious eliminated; this indicates a very good regulation of the work process. Another major advantage of the analyzed work installation is that SO_2 is not eliminated in the air as an exhaust pollutant; this consequence is associated with the burning of LPG in the steam generator.

As can be seen from the experimental research carried out, these automated equipments allow the rapid and permanent control of the temperatures of the steam jets, so that the noxious substances eliminated fall within the accepted limits.

The analyzes and experimental measurements performed at the output of the steam generator allowed the automated monitoring of the working parameters of the installation, in order to optimize the production process of different types of compound feeds. Also, the measured values for the concentrations of the pollutants eliminated in the air by the steam generator burner were within the provided technical limits, fully complying with European environmental protection rules.

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CONSTRUCTION MATERIALS ASSESSMENT TOWARDS SUSTAINABLE EXPANSION OF THE LEBANESE CITIES

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Abstract

Global annual resource use reached nearly 90 billion metric tons in 2017 and may even double by 2050. Since materials' extraction cause ± 8% of energy consumption worldwide and will increase due to resource scarcity, scientists search for equivalent sustainable materials. To decrease the materials' extraction and its negative effects, e.g. landscape degradation and ecosystem pollution, recycled and renewed materials are considered sustainable alternatives to decrease energy consumption. Thus, all countries including our case, a Mediterranean country importing most of its construction materials, should start exploiting locally available sustainable materials to address various environmental, social, and economic problems. Besides concrete, which is regularly used in Lebanon, Green Councils recommend the integration of many sustainable alternatives, e.g. straw bales, bamboo, recycled plastic, wood, rammed earth, ferrock and timbercrete. In this research, we analyze the potential of development for each alternative and its advantage to the Lebanese Community, with a focus on recycled plastic and chicken feathers due to their high availability in the country. After conducting interviews with sustainability specialists, the recommendations are to improve the concrete sustainability index since it is the most used construction material. Earlier studies have shown that concrete mixed with up to 3% chicken feathers makes it more sustainable. Recycled Plastic may be used alone or as an additive in concrete mixes. As a conclusion, recycled plastic construction materials and chicken feathers as sustainable additives to concrete mixes present the highest potential to be developed locally for their integration, and their immediate effect in limiting the fast expansion of dumping areas in Lebanon.

Keywords: *Sustainable materials, construction, environment, sustainability, Lebanese industry.*

Introduction

Countries worldwide started to realize the magnitude of the threats caused by environmental pollution endangering human health. Driven by the need to face such global threats, Green Building Councils were established to lead the transformation of the built environment to make it healthier and more sustainable, thus achieving environmental, economic, and social goals on a global scale. Rules, regulations, and impactful green building programs of work were published to control the construction development, to drive positive changes, and to reduce negative consequences of resource extractions that consume ± 8% of energy consumption worldwide (Olivetti.E et al., 2018). Environmental scientists started to investigate sustainable alternatives to contain these consequences that could double by 2050 if no serious measures were adopted. This paper will address the particular case of Lebanon, a Mediterranean country heavily dependent on importing construction materials. Particularly, we will study the case of the most used construction material which is produced locally:

Concrete. To control the concrete raw materials extractions, some researchers started exploring alternative resources while others worked on improving the concrete sustainability index by mixing it with recycled and renewable materials.

Lebanon is facing serious environmental pollution due to the presence of more than 300 open dumpsites characterized by open burning practices (Merhebi.F et al., 2014). These are mainly uncontrolled landfills, illegal garbage dumpsites in forests, remote areas, and near rivers. Those dumps do not only contain unusable trash, but also many recyclable materials that could be used for construction such as plastic, glass, and even some animal waste. In addition to soil and water pollution, Lebanon is facing the danger of desertification due to the quarries destroying its green mountains to extract limestone dust and other aggregates to be used in the production of Portland cement, which is the major component of concrete mixes.

This paper will evaluate a variety of alternatives and sustainable materials that can be used in construction to strongly limit the negative consequences of resource extraction on the environment in Lebanon. Instead of dumping unused plastic, glass, or even poultry feathers, recycling and renewing these wastes create an opportunity to boost the reduction of pollution by introducing more sustainable materials for exploiting in construction. However, besides plastic and poultry feathers, the use of alternative construction materials in Lebanon is limited due to the lack of production of raw materials, e.g. due to the unsuitable climate for bamboo. These alternatives, locally available on a smaller scale, can be introduced in the construction of specific projects which are targeting to achieve GREEN Certifications by increasing their sustainability and environmental value, e.g. residential and commercial buildings, rooftop gardens, airports, hospitals, public parks, etc.

Recycled plastic proves to have a high potential and diversity of usage as a sustainable alternative for many materials that are currently being imported and used in the construction industry, e.g. plastic boards, plasphalt, concrete products additives, etc. Plastic is highly used in Lebanon and mostly dumped in landfills, creating very serious environmental hazards and endangering ultimately all Lebanese citizens.

Advantages of using poultry feathers as a sustainable additive to the concrete mixes used in the Lebanese constructions will be explored to present the possibility of increasing the concrete sustainability index, reducing the areas needed for dumpsites, countering desertification and destruction of Lebanese mountains as well as creating a source of extra income for Lebanese farmers.

Materials and Methods

The paper is divided into two parts: Theoretical and empirical. Both parts will contribute to finding out which concrete alternative material can improve the concrete sustainability index by weighing the advantages and the highest potential to be produced locally.

In the theoretical part, the sustainable concrete alternatives and improving the material itself are described and studied. As the local products are more ecological than the imported ones since the shipping energy is to be considered, the paper studied the sustainable materials considered as concrete alternatives, its availability in the country, and the potential of using it in future projects. For the materials that were studied before for economic and environmental purposes, quantities were determined in this part of the research, such as cement production by BLOM Bank (Chami.M et al., 2014) and plastic waste UNSD (Merhebi.F et al., 2014). Furthermore, the non-determined quantities were investigated in the empirical part.

In the empirical part, interviews were conducted with environmental engineers and other sustainability experts in the country to identify the currently most used materials in the market, the way to improve its sustainability index, and which alternatives could be produced locally. After explaining the goals of the research, which are examining the potential of

expanding the usage of more sustainable construction materials in Lebanon and defining the problems which can be solved by using innovative strategies, guidance were given based on their experience in the studied subject. The recommendation given by Dr. Ali Karaki (PHD in Mechanical engineering, Sustainable Development Expert), was to determine the volume of renewable materials such as poultry feathers and plastic waste to quantify the impact of its use in new constructions. Also, it was recommended to use those materials in the construction field as raw materials or additives for concrete to improve its performance and sustainability index.

Based on the above recommendations, researches and surveys were conducted to determine the actual amount of plastic being dumped and its effect on the landfill expansion. After pointing out the advantages of chicken feathers as an additive and how it can be used as reinforcement in cement-bonded composites (Acda.N, 2010), interviews were completed with different specialists in animal production to determine the mass of chicken feathers produced locally.

Results and Discussion

Most of the interviewed environmental engineers consider that concrete will remain the most widely used material worldwide. Accordingly, besides working on its alternatives, they recommend working on improving the concrete sustainability index until the alternatives become commonly used in the market. That could affect quickly improving the sustainability of concrete buildings while working on developing and producing sustainable alternatives. Using sustainable concrete alternatives will reduce but not completely meet the demand of this key material in construction. To determine the effect of the recycled or renewable materials that could be used to enhance the energy efficiency of the buildings by increasing the sustainability index of concrete, the volumes of the conventional raw materials used nowadays were quantified. The study will show the ratio of renewable materials that could be added to concrete to increase its sustainability index. It will further show if the recycled plastic in the country should be used as an additive for concrete or if there is a potential of producing other construction materials out of it. The assessment results of improving the concrete sustainability index, and the expansion potential of its sustainable alternatives such as straw bales, bamboo, recycled plastic, wood, rammed earth, ferrock and timbercrete are described below (See table 1).

Since no studies were conducted before to determine the average quantity of concrete used yearly in Lebanese constructions, an investigation on cement production, the main composite of concrete, was done to determine the yearly concrete usage in the country based on the most used concrete mix. The investigations showed that the Lebanese Portland cement average production is 5 Million tons per year and around 94% of it is being used in the local market after 2015 (Chami.M et al., 2014). Generally, the most usable concrete mix is 350-400kg Portland cement, 800kg of sand, 1.200 kg of crushed stones, and 150kg of water. The ratio of Portland cement is 15%, thus the quantity of concrete poured in Lebanon is approximately 31.3 million tons per year.

There are two sustainable solutions to reduce the amount of used concrete. First, the sustainability index can be increased by adding renewable and recycled material to the mix such as recycled plastic and chicken feathers. Second, the used concrete can be substituted in some specific constructions by sustainable alternatives.

Lebanon generates about 1.57 million tons of recyclable and non-recyclable waste per year containing approximately 12% of plastic, which is around 20.102 tons per year of it is recyclable. It was proven in various researches done by Delhi-based CSIR-NPL, Australia's Royal Melbourne Institute of Technology and many other institutes that recycled plastic could

be used in construction in different products such as plasphalt, i.e. Asphalt with recycled plastic, structure lumber, roofing tiles, and bricks or as an additive for concrete with a ratio between 1 to 5% (Khanna.K, 2019). For chicken feathers, the yearly waste adds up to 22.800 tons, which could be used as a concrete additive with a ratio up to 3% (Sharma.A, 2016). This application will improve the buildings energy efficiency and reduce the construction concrete mix weight without having any negative effect on its compressive strength (Sharma.A, 2016). Plastic waste is generally not recycled as of today, but being either thrown in open dumps by the sea or landfilled creating a significant pollution factor. Since both plastic and chicken features are generally remaining unused and disposed, using it as a construction material will solve two main problems: The expansion of landfill that constitutes to a major problem in the country contaminating the soil and also underground water on the one hand, and reducing the expansion of quarries exploration destroying the mountainous landscape on the other hand.

To outline the potential of manufacturing concrete alternatives locally and study the impact of using it, the availability of those components and its raw materials was studied.

As the climate of Lebanon is Mediterranean, bamboo and wood have a very slow growth rate. Also, Lebanon's area is insufficient to consider planting forests of trees and bamboo for mass production. Thus, investing in this sector will not be sufficiently economical to be used in construction.

Compound for rammed earth should have an ideal percentage between 45 to 80% of sand, 10 to 30% of silts, and 5 to 20% of clay which is available in the country (Abreu e Lima.P et al., 2016). It is only a matter of wide spreading the expertise of developing this type of constructions that could be valuable solutions mainly for single or double floor buildings existing in rural areas.

For straw bales, the material is also available but the actual production is applied on 9.600 hectares only, which is minimal compared to what the construction sector demands. Lebanon could produce 1.776.000 bale per year of 14.5 kg each (Whitman.C et al., 2011). This can build around 3.550 houses of 200m², but it could not be further developed since the country does not have sufficient agricultural space (Mikhael.M et al., 2016). The actual production could also be introduced in some of the lightweight sustainable development, such as rooftop gardens, which becomes a trend in the Lebanese coastal cities that nowadays appear to be concrete forests.

Ferrock is created from 60% iron dust which is normally discarded from industrial processes fly ash that is a coal combustion product, limestone and silica from the ground up glass (Mouli Prashanth.P et al., 2019). The coal combustion machinery is used by cement factories rarely (UNSD, 2019), thus fly ash is not available in sufficient quantities in the country. Industrial steel powder is not widely available in the Lebanese market since it is not a major industry. Since ferrock could not be produced from local raw materials and importing it would result in a decrease in its sustainability index, this material cannot be considered as a sustainable material in the local market.

Timbercrete is a blend of sawmill waste, cement, sand, binders and a non-toxic deflocculating additive (Vasantha kumar.M, 2019). Since sawmill is the basic material making the product sustainable and wood manufacturing and production is not a big sector in the country, it could not be sufficiently reliable as an alternative material. Nevertheless, it could be produced in the local market to be used in some constructions.

Table 5: Solutions to replace concrete in construction materials or improve its sustainability index in Lebanon

Sustainable Solutions	Material	Yearly Production	Advantages	Threats	Conclusion
Concrete additives	Chicken Feathers	22.800 tons	• Improve concrete thermal performance • Reduce quarries and raw materials extraction • Reduce the concrete mix weight	• Affect negatively concrete properties if used at a ratio higher than 3%	• Available quantities are less than 3% of the concrete used in the local market, thus it could be all used in this industry
	Recycled Plastic	20.102 tons	• Reduce quarries and raw materials extraction • Reduce concrete shrinkage effect	• High negative effect on the concrete compressive tensile and flexural strength	• Better not to use it as additive since it affects compressive tensile and flexural strength
Concrete alternatives	Straw Bales	1.776.000 bale	• High thermal insulation • Lightweight construction material that could be used on weak roof structures	• No expertise in straw bales constructions • Not suitable for all types of constructions	• Sufficient quantity for around 3.550 houses • Technicities to build this type of construction should be widespread
	Bamboo	ND	• Have high compressive strength and light weight • May be used in the construction of scaffolding, bridges and houses	• Unsuitable climatic conditions for massive production • Investment in production will not be profitable	• Could be produced and used with limited potential since it is not sufficiently economical
	Recycled Plastic	219.800 tons	• May be used in different forms such as Plasphalt (asphalt mixed with recycled plastic), structure lumber, roofing tiles and plastic bricks	• Weak knowledge in production of recycled plastic construction materials • Waste plastic is unsorted from the source	• Awareness and training should be inducted on a global scale to illustrate potential benefits of using recycled plastic
	Wood	ND	• Low heat conductivity • No waste resulting from wood. All parts can be reused	• Insufficient areas dedicated for trees as mass wood production	• Limited potential of usage as profitability is minimum
	Rammed earth	ND	• Raw materials are available in the country	• Market weak expertise in building rammed earth houses • Cannot be used for high buildings	• Limited potential, for max. 2 floors • Lack of technical know-how for this type of construction, thus need for training
	Ferrock	ND	• Reuse the waste discarded from the industrial process	• Unavailability of these materials in the Lebanese market	• To be reconsidered whenever steel industry improves
	Timber-crete	ND	• Reuse the wood production waste for construction • Good thermal insulation	• Limited availability in the country	• Not sufficiently reliable, but may be produced for usage in some types of construction

Conclusion

Chicken feathers and recycled plastic prove to have high potential when used in producing concrete additives and/or alternatives in the Lebanese market. Chicken feathers having a mass of 0.072% of that of the concrete, when introduced in concrete mixes as an additive in a ratio of 3%, may have multiple usages especially in eco-friendly projects, where sustainability is the keyword for the architect, owner, and builder. Recycled plastic however, presented lower benefits when used as an additive to concrete while giving better results and a promising future when introduced in other manufacturing processes and materials, i.e. Plasphalt (asphalt with recycled plastic), structure lumber, roofing tiles, and bricks.

Producing these sustainable materials locally will increase the sustainability index and benefit the environment of the country by reducing its pollution mainly manifesting in the widespread of random landfills as well as countering desertification by the quarries. The energy needed for the extraction of resources as well as the cost of waste management will be significantly reduced. Poultry farmers will also have an additional source of income by the use of chicken feathers as an additive for the mainly used construction material

Nevertheless, the management of this field requires technical expertise and professional knowledge that remains limited nowadays in Lebanon. Additional investments, awareness programs, and professional training must be initiated by the government and the private sector to push towards the implementation of these sustainable processes until they become a norm in the sustainable expansion of the Lebanese cities.

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BIODEGRADABLE WASTE IN FRUIT AND VEGETABLES FACTORIES

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Abstract

Due to increasing demographic growth, industrialization, urbanization and economic development, the demands of the population for food and, therefore, food production are increasing, which is why large quantities of biodegradable waste are generated. Biodegradable waste is a potential energy source using modern processing technologies - biogas that would replace current commonly used fossil fuels (coal, oil, nuclear energy, etc.). In most food plants in Serbia, including one in Jarmenovci village in the municipality of Topola, the problem of what to do with the large amount of biodegradable waste that occurs during the processing of fruit and vegetable waste is expressed, because most food plants in Serbia were built 50 years ago or more, and then biodegradable waste was not viewed as energy. In this experiment, the analysis of biodegradable waste generated in the fruit and vegetable processing plants in Jarmenovci village was carried out. The scientific aim of this paper is to contribute to pointing out the problems of generating large quantities of biodegradable waste and to propose a solution for biodegradable waste.

Key words: *biodegradable waste, energy, processing*

Introduction

Since 1987, the world's population has increased by more than 1.7 billion. FAO's projections are that the earth's population will amount to 10 billion over the next 50 years. In many parts of the world, natural resources are used as basic livelihoods such as fisheries, forestry,agriculture.

The environmental impact of the food industry is reflected in the effects of the atmosphere, soil, flora and fauna and water. Climate change, such as excessive gas emissions, has led to an increase in the average temperature on earth, which results in melting glaciers, rising sea levels, the occurrence of droughts, storms, floods. Population growth and accelerated social development have led to intensive food production and the generation of large amounts of biodegradable waste. In the 1980s, at the world level, the average yield per acre of arable land was 1.8 tons, today that yield is 2.5 tons. Such intensive land use characterizes examples of modern agro-technical measures (Byjиħ, 2014).

Today, the food industry uses the most non-renewable energy sources, which are becoming increasingly scarce. The biodegradable waste that is generated during the technological processing process is not negligible, as it involves large quantities of bio-waste that can be used as energy (biogas) or compost by treatment (Moller, 2016; Jilani, 2004; Jovičić 2009).

The Biogas project is a complex mechanism involving various actors: biomass suppliers (farmers, food industry, companies, utilities), banks, investors, equipment suppliers, government and government services, and the public that can influence the development of biogas projects. The use of biogas for energy production requires long-term incentive mechanisms and the application of biogas in all sectors (electricity, heat, natural gas network, transport), as well as the promotion of these technologies (Byjиħ, 2014).

Composting, with recycling, is on the second level in the solid waste management hierarchy, below waste prevention and reuse prevention (Калабић,2005).
The EU Landfill Directive 1999/31 / EC, which bans the disposal of biodegradable waste into landfills, stimulates composting and other methods of third biodegradable waste, as a very convenient way of reducing the landfill of biodegradable waste. Depositing biodegradable waste at landfills results in undesirable emissions of both landfill gas and process water (Waste Framework Directives 2008/98/EC, 2009/28/EC and Landfill Directive 1999/31/EC).
The application of composting in Europe largely began in the late 1980s, in parallel with the extension and implementation of a separate bio-waste collection scheme. Aerobic composting is more prevalent because anaerobically requires higher investment costs and the need to treat wastewater as well. The Serbian economy has faced many challenges in the last 10 years related to property transformation and the global economic crisis. It should be especially emphasized that the Republic of Serbia has a large trade deficit in the import of energy and energy production, where projects using biogas in energy production can help reduce it. The use of biogas for energy production involves investments in various activities. The investment climate in Serbia is unpredictable and a long period is needed for the entire procedure of building a biogas plant. Also, the overall investment rating of the country is low, and domestic banks are not ready to finance biogas projects in Serbia due to increased economic risk (Byjић, 2014).
For the purposes of this research, the Jarmenovci Fruit and Vegetable Processing Plant was selected as a plant that generates large quantities of biodegradable waste during its operation. Therefore, the idea arose to calculate what amount of waste and where it is generated during the technological process, based on the monitoring of one processing season.
The conducted research provides basic data on the amount of biodegradable waste, but in the future it is necessary to use that waste as fuel to initiate the recycling process itself, which is also a goal of master work. The scientific aim of this paper is to contribute to pointing out the problems of generating large quantities of biodegradable waste and to propose a solution for biodegradable waste.

Material and method

The Jarmenovci factory carries out the activity of processing fruits and vegetables at the location in Jarmenovci, Topola Municipality, as well as a refrigerator for deep freezing and storage of raw material used in further processing or sold as such.The village of Jarmenovci is located in a very fruitful area, there are conditions for the production of vegetables, so the orientation of this factory to hot and cold processing of fruits and vegetables was technologically justified.Biodegradable waste is a type of waste of organic nature, substance that can be decomposed naturally, that is, by chemical reactions over a period of time whose length depends on the composition of the substance itself.This paper will show the quantities of biodegradable waste for the three most important products (cherry, plum and pepper) processed at the Fruit and Vegetable Processing Plant in Jarmenovci for 2018 and 2019.
The technological operations of processing cherries,plums and pappers can be divided into three stages:

- Preparation for freezing(washing, calibration, boneless sour, surface drying)
- Freezing in a continuous tunnel (-20ºC)
- Packed frozen fruit in boxes 10/1

Results and disscusion

Table 1. Biodegradable cherry processing waste, pitted cherry processing in 10 kg boxes in 2018 and 2019.

Cherry form	Quantity[kg] 2018 Year	Quantity[kg] 2019 Year
Fresh cherry	1 024 014	1 039476
Cherry , pitted chery10/1	1 664 087	1 695 310
Waste cherry p.c.	65 437	12 437
Bone	92 162	95 552
Pieces of meat on the bone	92 162	42 579
Total biodegradable waste	133 122	136 131

The total amount of biodegradable waste obtained by processing frozen pitted cherry into 10 kg boxes for 2018 and 2019 is 269 253 kg (Analysys of labaratory " In Vitro" - Šabac)

Table 2. Biodegradable plum processing waste, Processing of plum machine-cut hemisphere in 10 kg boxes in 2018 and 2019.

Plum shape	Quantity[kg] 2018 Year	Quantity[kg] 2019 Year
Fresh plums	513 519	294 674
Plum way out.10 / 1	387 530	210 310
Bone	35 948	19 562
Pieces of meat on the bone	10 272	5 605
Total biodegradable waste	46220	25167

The total amount of biodegradable waste obtained from processing frozen plums of machine-cut hemisphere into 10 kg boxes for 2018 and 2019 is 71 387 kg.
It is necessary to perform a qualitative analysis of waste, microbiological analysis, heavy metal content and physical and chemical properties. In order for biodegradable waste to be safe and favorable for the production of compost or biogas, the obtained analysis results should be negative.

Table 3. Biodegradable plum processing waste, Processing peppers in boxes of 10 kg in 2018 and 2019.

Pepper form	Quantity[kg] 2018 Year	Quantity[kg] 2019 Year
Fresh peppers	459 532	578 112
Frozen peppers	262 300	318 510
Total biodegradable waste	128 670	161 872

The total amount of biodegradable waste obtained from processing frozen peppers into 10 kg boxes for 2018 and 2019 is 290 542 kg.

Analysis of the state of use of biogas in the Republic of Serbia

Energy production on waste is a process environmentally, economically and socially sustainable and has strong potential to produce energy from communal and industrial waste. By exploiting the energy potential of municipal solid waste the country can ensure sustainable development as well as energy security (Bajic at al., 2015).
The energy sector is the largest economic sector and has a significant economic role in the Republic of Serbia, with a share of more than 10% in gross domestic product (GDP). Total

primary energy consumption in Serbia was 16.19 Mega tonnes of oil equivalents (mten) in 2011, of which domestic production was 62% and imports was 38%. Domestic production of primary energy involves the exploitation and use of indigenous resources of coal, crude oil, natural gas and renewable energy sources (hydro, wood biomass, geothermal energy, etc.) (NREAP 2013)

Climate change, mainly driven by an increase in emissions from the energy sector, as well as a reduction in fossil fuel reserves, has initiated a number of global mitigation measures. One of these measures is to increase the use of renewable energy sources, as defined in European Union Directive 2009/28 / EC. The Republic of Serbia, as a candidate for EU accession, has pledged to implement EU principles and implement concrete measures to support the production and use of "green" energy.

In the Republic of Serbia there are two priorities related to renewable energy sources:

• Establishing a stable and stimulating regulatory framework for renewable energy.

• Development and construction of new energy infrastructure facilities (power grid power plant) for the production of energy from renewable energy sources, its transmission and distribution (NREAP 2013).

In the process of EU accession, the Republic of Serbia has accepted the obligation to implement all EU directives related to renewable energy sources. Serbia has also committed itself to increasing the share of renewable energy in total final consumption from 21.1% in 2009 to 27% in 2020. In order to achieve this goal, the Republic of Serbia has prepared an Action Plan for renewable energy by 2020. According to this plan, the Republic of Serbia should have 30 MW of installed biogas power plants by 2020 (SEPA 2012).

In the Republic of Serbia there is no register of production potential of biogas from different raw materials. Through a comparative approach to the analysis of biogas production potential from the most important resources, as well as to the analysis of existing economic and administrative barriers to biogas use, the aim of this paper is to contribute to laying the foundations that will be used to identify real and economically viable production and deployment potentials biogas in the Republic of Serbia.

Based on the analysis of previous biogas production potential studies presented in the introductory chapter, agricultural crops, livestock waste streams, communal waste, meat and milk industry waste streams were used as sources for which biogas production potential was determined.

Conclusion

Projections accelerate population growth and carry and disperse the world's economies, and time and increase environmental pollution. The basic role of the food industry provided sufficient quantities of food for the population. The trend of population growth on the land is needed for the ever more intensive production of food, which is conditioned by the already first agricultural means. Environmental protection is also of great importance for the food industry, which generates waste through three cycles of waste disposal, in agriculture, processing and resale. Most biodegradable waste is generated which could be used as energy for its own energy needs.In the future, the food industry will have to dispose of waste as a product of value in order to compete with the European food industry that uses its waste (recycling, composting, biogas production, etc.).Fossil fuels are a finite resource and contribute to the greenhouse effect. Biogas is a renewable resource and helps protect the climate as well as protect scarce resources.

Environmental protection, as well as energy conservation, is gaining importance, and from that point of view, the construction of anaerobic digesters has great potential. At the level of state institutions, there should be support for this type of energy production and use. That

support would be reflected in various incentive measures, such as subsidizing the production and use of equipment for the production and use of biogas in the food industry. Since Serbia is in the process of joining the European Union, it is extremely important to develop an adequate system of waste management together with the development of society and economy as a whole.

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CLIMATE CHANGES AND FIRES IN BOSNIA AND HERZEGOVINA

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Abstract

Like most countries in the world, Bosnia and Herzegovina (BiH) is facing the consequences of climate change. There are catastrophic floods on the one hand and extreme droughts on the other, especially during the last decade. In addition, there is an increasing number of fires, especially in the Herzegovina region. Weather and climate, vegetation condition and composition, and human factors play an essential role in fire regimes. Higher average temperatures and less precipitation during the summer increase the risk of fire. As a main component of risk, wildfire danger is linked with the factors, including weather and climate, which can worsen either the likelihood of ignition, or the behaviour of the fire once ignited. Considering climate change scenarios, it is very likely that the damage they cause will be even greater in the future.

Keywords: *Climate changes, fires, Bosnia and Herzegovina*

Introduction

Bosnia and Herzegovina has several climate types: the temperate continental climate type (northern and central parts), the sub-mountainous and mountainous type, the Mediterranean and modified Mediterranean climate type. It has a relatively preserved environment since the rural area dominates and 46.34% of the territory covered by forests.

Estimates show that BiH will be exposed to climate change impacts that could have consequences for its entire society. Studies of temperature change for the period 1961-2010 indicate that temperatures have increased in all areas of the country. A comparative seasonal analysis for 1981-2010 and 1961-1990 showed that the largest increases in average temperature during the summer months were observed in Herzegovina (Mostar 1.2°C). The largest decrease in annual precipitation was during the spring and summer seasons, in the region of Herzegovina (Mostar and Bileća, up to 20%). Pronounced variability in the annual rainfall regime and temperature increases are key factors in the occurrence of more frequent and intense droughts in Bosnia and Herzegovina (SNC, 2013).

Analysis of meteorological data from the period 1961-2014 show that the mean annual temperature maintains a continuous rise. The increase in annual air temperature ranges from 0.4 to 1.0°C, while the increase in temperature during the growing season (April-September) even reaches 1.0°C. The analysis of the index of temperature extremes confirms the increasing frequency of extreme maximum temperatures whereas the frequency of extreme minimum temperatures is lower (TNC, 2016). The hottest part of the country is area Herzegovina lowland, where mean annual temperatures have an average value from 11 to 14°C, with areas of Mostar, Trebinje and Popovo field over 14°C.

Results from two global climate models: SINTEX-G and ECHAM5 indicate a mean seasonal temperature increase averaging +1°C by 2030 compared to the base period 1961-1990 over the whole Bosnia and Herzegovina. The largest increase of +1.4°C is expected during summer time (June-August). For the A2 scenario (2071-2100), the rapid temperature increase of +4°C yearly average is expected, while the expected increase in temperature during summer time

will go up to +4.8°C. Models indicate uneven precipitation changes. A slight increase in precipitation in mountain and central areas is expected, while negative precipitation anomalies are projected for the other areas. According to the scenario for the period 2071-2100, negative precipitation is expected across the whole BiH territory. The largest precipitation deficit of up to 50% compared to the base period 1961-1990 is expected during summer months (SNC, 2013). Weather and climate are considered to be among the main factors influencing wildfire potential and define the composition and structure of vegetation fuels. Although human activities and vegetation management shape the actual state of vegetation fuels, their typology is subject to the general ecological domains in which they exist. Additionally, weather effects can strongly influence the susceptibility to fire of vegetation fuel. Weather can control the moisture content of the vegetation, allowing a rapid wetting or drying of fine fuel (litter, needles, mosses, twigs), while having a slower response on coarser wooden fuels. The moisture level in these different fuels, along with weather factors such as wind speed, affect the ease of ignition, potential propagation and severity of a fire (Costa et al, 2020).

According to FAO (2015), major reasons for fires in BiH are: no active fire management, high amount of fuel wood in forest; low capacities and bad equipment for fire-fighting, mostly dependent on support from neighbouring countries (e.g. from Croatia); ignorance about forest fire risks and prevention measures; carelessness of local population and tourists.

There are no valid and official data for the main causes of forest fires in BiH, although unofficially the main cause is the human factor (in about 98 percent of all forest fires). According to some unofficial sources, the main reasons for forest fires in BiH are agricultural burning (field clearing in spring and stubble burning in summer) and negligence when lighting fires in or near forests. There are some cases of arson, but these are not proved as there is no official investigation or court verdict. Lightning is a minor cause of forest fires (fewer than 2 percent of cases) (RFMC, 2015).

Material and Methods

For analysis impact of climate changes on fires in BiH during the period 2000-2020 national reports under the UN framework convention on climate changes is used, data from meteorological institutes, institutes for statistical data, etc. The main information source about fires was CORINE[2] Land Cover (CLC) database, European Forest Fire Information System (EFFIS) and Global Wildfire Information System (GWIS). The CLC is a project launched by the European Environment Agency (EEA) more than thirty years ago with the aim of collecting, coordinating and ensuring the consistency of information on natural resources and the environment. The first CLC project for Bosnia and Herzegovina started in 1998 and was successfully completed in 2000. The result was the creation of the BiH CLC 2000 database, which included the identification of the types of surface cover at the level of the main classes, and also the second and third level subclass with a detailed description of the structural characteristics. Subsequently, the CLC 2006, CLC 2012, and CLC 2018 databases were created with the aim to monitor the dynamic changes in the land cover (Drašković et al, 2020). CLC nomenclature comprises three levels: the first level (5 items) indicates the major categories of land cover on the planet, the second level (15 items) is for uses on scales of 1:500,000 and 1:1,000,000, and the third level (44 items) is for uses on a scale of 1:100,000 (EEA, 1995).[3] The analysis of CLC data and land cover changes data was done by extracting the territory of Bosnia and Herzegovina using QGIS 3.12.3 (2020) clip tool and exported to

[2] CORINE-COoRdination of INformation on the Environment.

[3] For detailed information about CLC nomenclature: https://land.copernicus.eu/user-corner/technical-library/corine-land-cover-nomenclature-guidelines/html

Microsoft excel worksheet. The data was sorted and summarized using SUBTOTAL SUM tool by land cover types and changes for all levels and periods.
EFFIS was created through cooperative effort of the countries and the European Commission Services, and is open to all European countries. The system became operational in 2000. Since the year 2015, EFFIS is part of the EU Copernicus Program, under the Emergency Management Service (EMS) (source: https://effis.jrc.ec.europa.eu/about-effis/brief-history/). Through its website, EFFIS provides fire danger predictions up to six days in advance of their occurrence, as well as daily updated information on active fires and fire damages in Europe (source: https://ec.europa.eu/jrc/en/research-topic/fires).[4]
GWIS is a joint initiative of the GEO and Copernicus Work Programs. GWIS aims at bringing together existing information sources at regional and national level in order to provide a comprehensive view and evaluation of fire regimes and fire effects at global level and to provide tools to support operational wildfire management from national to global scales (source: https://gwis.jrc.ec.europa.eu/).

Results and Discussion

Analyzing the periods 2000-2006, 2006-2012 and 2012-2018, CLC database shows increasing number of fires and burnt areas. During the first period, number of fires was 14 and burnt areas 1046 ha in total. 54 % of fires are related to broad-leaved forest (Table 1). Burnt areas participate with 2.2 % in total changes.

Table 1: Fires in BiH 2000-2006.

Changes	Land cover type	Number of fires	P (ha)	P (% fires)	P (% of total changes)
3.1.1-3.3.4	Broad-leaved forest	4	568	54	1.2
3.1.3-3.3.4	Mixed forest	1	30	3	0.1
3.2.3-3.3.4	Sclerophyllous vegetation	2	173	17	0.4
3.2.4-3.3.4	Transitional woodland/shrub	4	252	24	0.5
3.3.3-3.3.4	Sparsely vegetated areas	1	22	2	0.0
	Total	12	1046	100	2.2

Period 2006-2012 recorded 28 fires and 2520 ha of burnt areas which is more than doubled comparing to first period (Table 2). Broad-lived forest is again most affected with 48% of total fires. Percentage of burnt areas in total changes increased to 12.7 % comparing to 2.2% in the first period.

Table 2: Fires in BiH 2006-2012.

Changes	Land cover type	Number of fires	P (ha)	P (% fires)	P (% of total changes)
3.1.1-3.3.4	Broad-leaved forest	6	1221	48	6.2
3.1.2-3.3.4	Coniferous forest	6	306	12	1.5
3.1.3-3.3.4	Mixed forest	3	438	17	2.2

[4] EFFIS normally operates using meteorological forecast data received daily from 3 systems, the European Centre for Medium-Range Weather Forecast (ECMWF) and French (MeteoFrance). Fire danger is mapped in 6 classes (very low, low, medium, high, very high and extreme) with a spatial resolution of about 16 km (ECMWF data), 10 km (MF data) and 36 km (DWD data) (EFFIS User Guide, 2018).

3.2.3-3.3.4	Sclerophyllous vegetation	3	290	12	1.5
3.2.4-3.3.4	Transitional woodland/shrub	9	252	10	1.3
3.3.3-3.3.4	Sparsely vegetated areas	1	13	1	0.1
	Total	28	2520	100	12.7

Period 2012-2018 has almost 4 time more fires comparing to first period, with 80 fires and 8521 ha of burnt areas. Most affected were sclerophyllous vegetation (3.2.3-3.3.4) with 9.8%, transitional woodland/shrub (3.2.4-3.3.4) with 8.7%, and broad-leaved forest (3.1.1-3.3.4) with 3.7%. Fires caused 22.6 % of total changes (Table 3). So that, in some areas, the current vegetation structure might become irrecoverable after fire damage.

Table 3: Fires in BiH 2012-2018.

Changes	Land cover type	Number of fires	P (ha)	P (% fires)	P (% of total changes)
2.3.1-3.3.4	Pastures, meadows and other permanent grasslands	1	166	2	0.4
3.1.1-3.3.4	Broad-leaved forest	22	1410	17	3.7
3.1.2-3.3.4	Coniferous forest	1	8	0	0.0
3.2.3-3.3.4	Sclerophyllous vegetation	22	3676	43	9.8
3.2.4-3.3.4	Transitional woodland/shrub	34	3261	38	8.7
	Total	80	8521	100	22.6

Of the 100% of fires during the period 2000-2018, about 70% belong to the period 2012-2018, 21% to the period 2006-2012 and 9% to the period 2000-2006. All the recorded fires were in the south of the country, in the region of Herzegovina, known for its Mediterranean climate with high temperatures and low rainfall during the summer season (Figure 1). Most affected zones are within Trebinje city local area and Herzegovina-Neretva canton. The main fire season is summer.

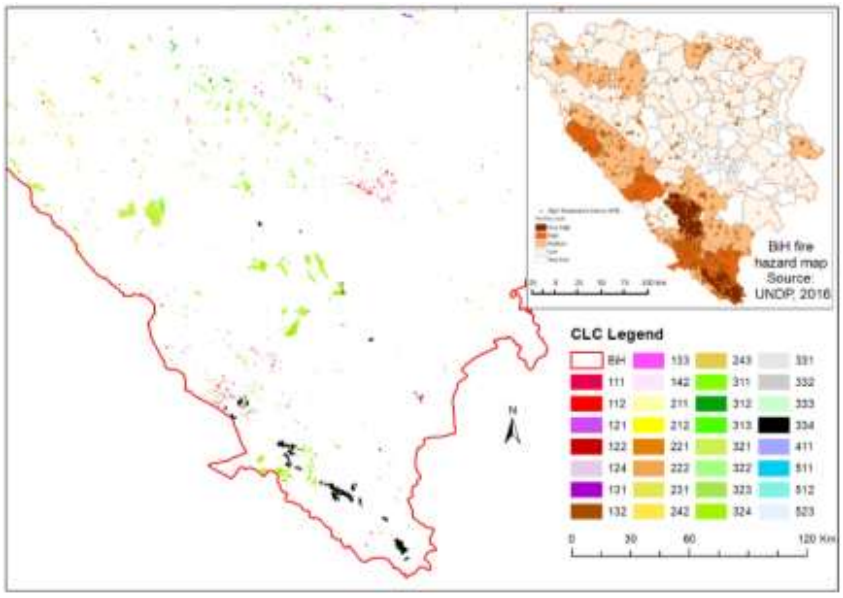


Figure 1: CLC changes 2000-2018: Burnt areas at Hercegovina region (code 3.3.4 - black colored)

In Mediterranean climes, terpenoids play an important role in wildfires and vegetation dynamics. They are present in conifers and in several sclerophyllous plants rich in essential oils, increasing their flammability rate. When in high concentration in litter, terpenoids also inhibit seed germination. Wildfires, by destroying these substances accumulated on the ground, promote the colonisation of new species, including the germination of seeds from the same plant that originally produced the terpenoids, which needs fire for its regeneration (San-Miguel-Ayanz et al., 2016).

The new vegetation may be quite different from the original. Several stages of ecological succession may follow the first vegetation regrowth, with variable temporarily dominant species (and potentially worse temporary fuel characteristics) until the original ecosystem is recovered. Therefore, even a grassland may require several years to re-establish the balanced ecosystem functions and services provided before the fire. The example applies even more demandingly to forest ecosystems (Costa et al., 2020).

The largest individual changes related to fires were recorded in the transition of burnt areas to natural grasslands (3.3.4-3.2.1) with 16.4% (just one year after a fire, a grassland may appear to be green again) of the total changes, burnt areas in transitional woodland/shrub (3.3.4-3.2.4) with 14.8%. Areas with sparse vegetation (3.3.4-3.3.3) recorded a recovery of 3.05%, while other burned areas such as vineyards, deciduous forests and sclerophilous vegetation recovered slightly (Table 4).

Table 4: Recovered areas after fires in BiH 2000-2018.

Period	Changes	Land cover type	Recovered areas	P (ha)	P (%)	P (% of total changes)
2000-2006	3.3.4-3.2.4	Transitional woodland/shrub	1	70	100	0.1
	Total		1	70	100	
2006-2012	3.3.4-3.2.4	Transitional woodland/shrub	1	26	100	0.1
	Total		1	26	100	
2012-2018	3.3.4-2.2.1	Vineyards	1	14	0	0.0
	3.3.4-3.1.1	Broad-leaved forest	1	73	1	0.2
	3.3.4-3.2.1	Natural grassland	2	6166	47	16.4
	3.3.4-3.2.3	Sclerophyllous vegetation	3	158	1	0.4
	3.3.4-3.2.4	Transitional woodland/shrub	14	5575	43	14.8
	3.3.4-3.3.3	Sparsely vegetated areas	2	1020	8	2.7
	Total		23	13006	100	34.5

Generally, changes related to burnt areas (3.3.4) dominate, accounting for more than half of all changes in the period 2012-2018 (56.73%), in both directions. This shows that fires were the most significant factor influencing the dynamics of spatial changes in this period and they significantly affect the land cover types in Bosnia and Herzegovina.[5]

[5] According to National report CLC2018 the biggest change is transition of burned areas into forest and semi-natural areas on the surface of 53.7% of total changes. Deforestation (3.1.x to 3.2.4) affected 16.3%. At the same time only 4.6% of the land were converted to forest (CLC2018 BA, 2019).

EFFIS recognize fires as the most recurring hazard in Bosnia and Herzegovina.[6] The occurrence and propagation of fires depend on many factors, some of them natural and others related to human activity. Climate is one of the most important factors influencing wildfire risk and climatic conditions can enable fires to take hold and spread quickly. A decrease in precipitation during the summer affect the reduction of vegetation water content. BiH is considered as country with high frequency of days with high fire danger (FWI>30)[7] and very high number of fires for the period 2000-2017.

Figure 2 shows overview of total burnt area for the years 2008-2020 from satellite images of fires of over 30 ha mapped by EFFIS.[8] There are increasing number of fires and burnt areas. 2012 and 2017 were particularly affected, while 2020 only in the first 6 months has extremely high values of burnt areas due to spring burning in agriculture.[9]

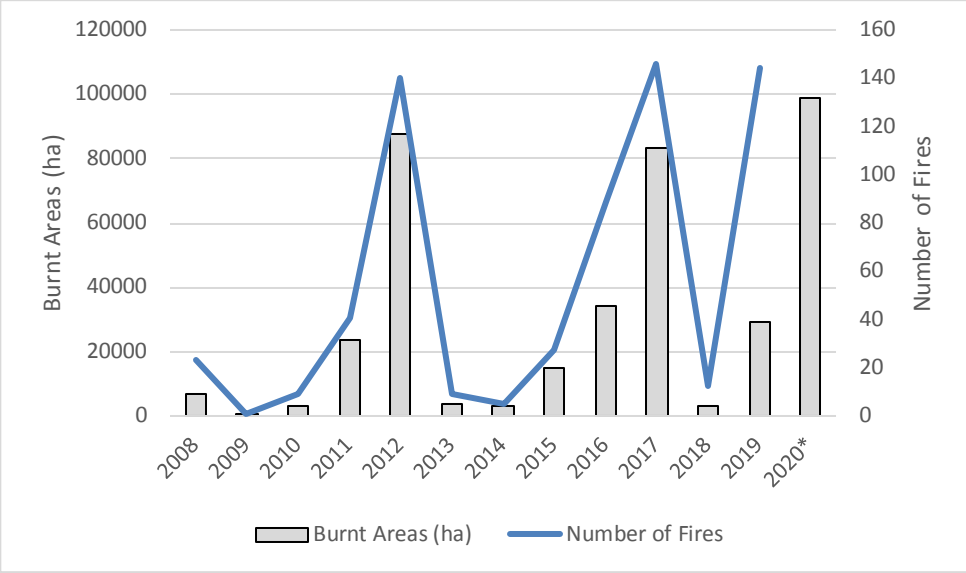


Figure 2: Annual country statistics for BiH (Source: EFFIS, 2020. *Data for period January-June of 2020)

For the first five-year period (2008-2013), burnt areas cover 125066 ha and number of fires was 223. In the second five-year period (2014-2019) burnt areas increase to 167924 ha and number of fires is almost doubled with 423. Annual average of burnt areas since 2008 is 24416 ha and fires number is 54.

GWIS data for BiH refer to the period from 2001 to 2018. Annual average of burnt areas according to this database is 19902 ha and number of fires is 67 (GWIS, 2020). The EFFIS and GWIS data are partially different but shows a similar trend.

Table 5 shows that the most of burnt area belongs to forest or other natural land, agricultural land is much less endangered and other land cover almost none.

[6] BiH is included in EFFIS country statistic since 2008 as a European non-EU country.

[7] Fire Weather Index (FWI) is used to estimate numerically the weather-driven fire danger.

[8] Only 3.6 % of the wildfires recorded by the Fire Database of EFFIS have an area burnt greater than 30 hectares. However, they contribute to more than 79 % of the total burnt area. Larger fires are even less frequent, but their damage is outstanding (Costa et al, 2020).

[9] Seasonal trend for BiH shows rapid increase in period 11th march to 15th April 2020, with 97897 ha of burnt area comparing to 7014 ha average for period 2008-2019.

Table 5: Distribution of burnt area (ha) in Bosnia and Herzegovina by land cover types over the period 2010-2018

Year	Artificial Surfaces		Agricultural Areas		Forest/Other Wooded Land		Other Natural Land		Other Land Cover		Total
	Area burned	%	Area burned	%	Area burned	%	Area burned	%	Area burned	%	
2010	0	0.0	177	5.3	955	28.5	2218	66.2	0	0.0	3350
2011	37	0.2	1404	8.2	10276	60.1	5369	31.4	14	0.1	17100
2012	4	0.0	10480	12.0	51275	58.5	25931	29.6	7	0.0	87697
2013	0	0.0	290	8.4	2560	74.0	608	17.6	0	0.0	3458
2014	0	0.0	48	1.5	508	15.8	2648	82.7	0	0.0	3204
2015	0	0.0	1999	13.3	4700	31.3	8332	55.4	0	0.0	15031
2016	0	0.0	5739	16.6	11141	32.3	17601	51.0	1	0.0	34482
2017	17	0.0	5710	6.9	45055	54.2	31693	38.1	660	0.8	83134
2018	0	0.0	492	15.7	1982	63.2	654	20.8	11	0.4	3139

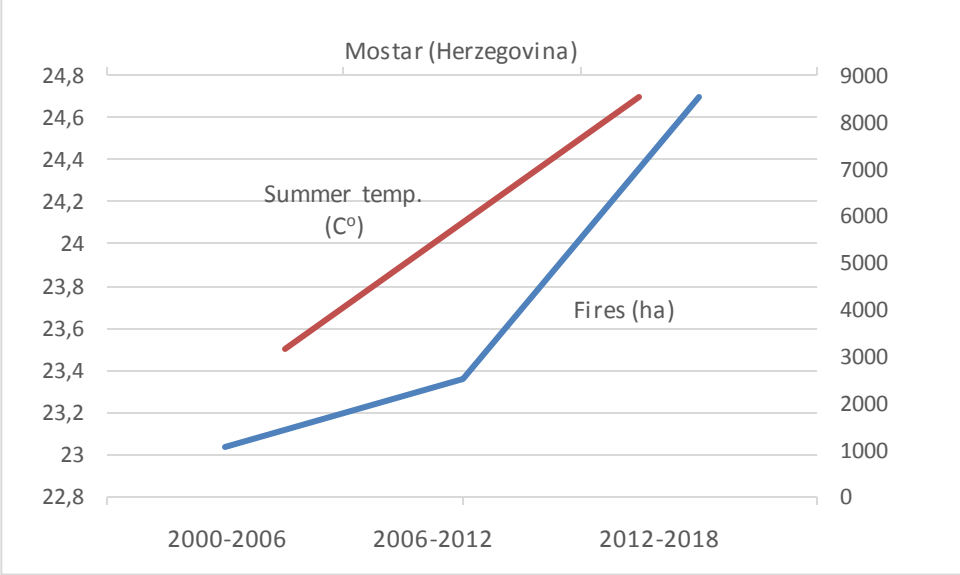


Figure 3: Correlation between change in air temperature (°C) in Mostar (Herzegovina, 1961-2010) and burnt area (ha) over the period 2000-2018.

Conclusions

Climate change may directly change fire regimes and affect the biophysical conditions of ecosystems. Natural conditions that affect moisture content and fuel properties are very important in the ignition and propagation of fires. Region of Herzegovina is the most exposed to climate changes and fire risk due to largest increases in average temperature (up to 1.2 ^{o}C) and decrease in precipitation (up to 20%) during the summer months. According to CLC database, percentage of burnt areas in total changes rise from 2.2% in period 2000-2006, over 12.7% during 2006-2012, to 22.6% in period 2012-2018. Also, EFFIS and GWIS database shows increasing trends in number of fires and burnt areas. The most endangered are forests and other natural land. Positive process is that the burned areas recover relatively quickly. In the period 2012-2018, it was the most intensive trend with 34.5% of total CLC changes.

Climate models indicate a mean seasonal temperature increase averaging up to +4.8°C for period 2070-2010 compared to the base period 1961-1990. Also, the largest precipitation deficit is expected during summer months up to 50%. Climate change will reduce fuel moisture levels from present values. Whole the country will become drier, increasing the climate-driven danger of forest fires.

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THE DEPOLLUTION OF THE WHEY REJECTED BY THE CHEESE INDUSTRIES USING CHEMICAL, PHYSICAL AND MICROBIOLOGICAL METHODS.

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Abstract

In most developing countries, the cheese industry releases thousands of liters of whey a day into the wild. This whey is considered a waste. It has great biological value but it is also very polluting and harmful to the environment. The aim of this study is to evaluate several methods of depolluting whey on a laboratory scale and extrapolate the results on a large scale: the industrial scale. First, the physico-chemical characterization of the whey harvested from *GIPLAIT Tlemcen* (Algeria) was carried out. Then, different treatments were realized on our samples: physical, chemical and microbiological. The measurement of BOD5 (via Oxitop) of the different samples was made before and after treatment in order to evaluate the depollution performance. The results showed that the deproteinization and de-sugaring by microbiological culture had the highest depollution efficiency, a decrease of the BOD5 that reached 29%. These results can be obtained by ultrafiltration, nanofiltration, reverse osmosis and bioreactor systems on an industrial scale. As a conclusion, the depollution of the whey goes through its de-sugaring. The best and easiest applicable methods of depollution/valorization are the drying/atomization and the ultrafiltration/nanofiltration.

Key words: *Whey, Depollution, BOD5*

Introduction

The dairy industry, especially the cheese industry, occupies a large place in the food industry market. Large quantities of milk are used for cheese production, which result in the release of large quantities of whey after production. This release impacts heavily the environment due to the high organic matter of whey which is a perfect environment for many microorganisms. Thus, making it the food industries waste with the highest BOD and COD.
Whey generates significant organic pollution: 1 liter corresponds to around 85% of the daily pollution generated by an inhabitant (Laplanche, 2006). Despite this, whey has great biological value. In several countries, it is used to obtain several other products such as lactic acid, bio-ethanol, dietary proteins… etc. This recovery or valorization allows partial or total depollution of the whey. Several studies have been discussed on this subject in Algeria, a country which neither values nor cleans up whey, such as Gana & al (2001) as well as Tebbouche (2012). Moreover, no action has been taken by the involved industries. In this study, authors assessed the performance of several methods for depolluting whey: physical,

chemical and microbiological methods while focusing on what is achievable and applicable on a large scale and on the Algerian cheese industry (or the classic cheese industry).

Materials and methods

Physico-chemical characterization of the harvested whey

To begin with, we obtained the whey from the *GIPLAIT Tlemcen* cheese factory (in february 2020) that rejects around 3,500 liters of whey per 5,000 liters of milk used to produce Swiss cheese. The whey obtained was stored in plastic drums under a temperature between 0 °C and 10 °C. After the harvest, the physico-chemical characterization was carried out. The measured parameters, which are the most significant and important tools in our study are: Water, Dry extract, Protein content, Lactose content, Fat content and Acidity (pH).

- Water and **dry extract** (by *lyophilization* and *infrared drying*):

Lyophilization: The whey was frozen for 24 hours under a temperature of -80 °C via a ***Zhongke Meiling HL100/218/290*** freezer. After that, the frozen whey was placed in the ***Christ Alpha 12 LD+*** lyophilizer, the temperature and pressure were adjusted and lyophilization started. 24 hours later, the whey powder was recovered and the pre and post lyophilization weighing were compared.

Infrared drying: A 5g sample was placed on an aluminum display stand which was itself placed on a ***Ohaus MB25*** desiccator. Infrared drying occurred. Once finished, the displayed percentage which refer to the water content was noted.

-Protein content (by *spectrometry /Biuret method*):

We carried out increasing dilutions of our sample (1/8, 1/16 and 1/32) and added to the batch the standard solutions based on gelatin (1 g/ L, 5 g/L, 10 g/L) as well as the blank (distilled water) (all volumes are 1 ml). 1.2 ml of Biuret reagent were added to all the solutions and then were dried for 30 min at 30 ° C. After incubation, the optical density of all the solutions was measured, starting with the blank in order to calibrate the spectrometer (the wavelength was adjusted to 540 nm). The results obtained for the experimental curve were then compared to the standard curve and the protein content of our sample was extrapolated.

-Lactose content (by *titration /Fehling method*):

We started by centrifuging 20 ml of our sample at 3000 rpm for 5 minutes. 10 ml of supernatant were recovered and added to 30 ml of methanol and left for 10 minutes (liquid/liquid extraction). Centrifuge was carried out for 10 min at 3000 rpm and the supernatant that contains the sugars were recovered. Two titrations were carried out simultaneously, with the same titrating solution (2 ml Fehling liquor A + 2 ml Fehling liquor B). For the titrated solutions, we had on one side our sample V1 (supernatant obtained after liquid/liquid extraction and centrifugation) and on the other side a control solution V2 (glucose solution at a rate of 5 g/L C2). The sugar content was obtained by the following formula:

$$[C2 = ((C1xV1)/V2)x5x4]$$

-Fat content (by *acid-butyrometric assay /Gerber method*):

In a Gerber butyrometer: 10 ml of whey were added to 1 ml of isobutyl alcohol and 10 ml of NaOH (6.5%). The mixture was heated to 60-65 °C for 4-5 min and then centrifuged (1500 rpm) for 4-5 min. Reading by pointing the base of the butyrometer upwards.

-Acidity or **pH** (by *pH meter*).

Depollution tests

Several depollution methods have been carried out, each method retains or excludes a category of molecules from our sample.

The parameter measured was BOD5 or biochemical oxygen demand after 5 days of incubation, this parameter was chosen for its speed and ease of application as well as for the unnecessity of using nitrification inhibitors such as allyl thio- urea).
The treated solutions were named as follows: L.D.P.S (deproteinized whey by saline precipitation), L.D.P.T (deproteinized whey by thermoprecipitation), L.D.L.A (de-sugared whey by alcoholic solubilization), L.D.P.L.M (deproteinized and de-sugared whey by microbiological culture).
Two control solutions were added to the measurement batches: Lac N (untreated whey /in order to measure the depollution yield), Glucose control (glucose solution /in order to attest the validity of the microbiological strains used for the measurement).
The data conversion formula used is as follows:
[DBO5 = Readed value*Table factor*Dilution factor] (this formula was designed according to the instructions presented in the manual directed by Tandia, 2007).
The BOD5 was mesured with an ***Oxitop*** device.
Figure 1 serves as a summary of the measurement protocol applied for the present study.

Results and discussion

Physico-chemical characterization

The results obtained (**Tab.1**) conform to the bibliographic references, however two parameters remain outside the batch:
The **lactose content***, which was 30% less than what was mentioned in the references, was due to the fact that the harvested whey was obtained by an acid coagulation process via lactic ferments, these same ferments had to consume lactose present in whey to produce lactic acid which lead to the coagulation of milk.
The **protein content****,which was 15% less than what was mentioned in the references, was due to the fact that the coagulation processes used to obtain the harvested whey were carried out at high temperatures thus leading to a low thermoprecipitation of the proteins found in the product of the milk coagulation (Swiss cheese).

Tab.1 Physico-chemical characteristics of the harvested whey.

	Harvested whey (%)	**Acid whey (%) (Morr & al. 1993 ; Linden & al. 1994)**
Water	**93,99**	**93,5**
Dry extract	**6,01**	**6,5**
Lactose	**3,1***	**4,71**
Proteins	**0,5****	**0,75**
Fats	**0,05**	**0,03**
Acidity (pH)	**4,57**	**4,6**

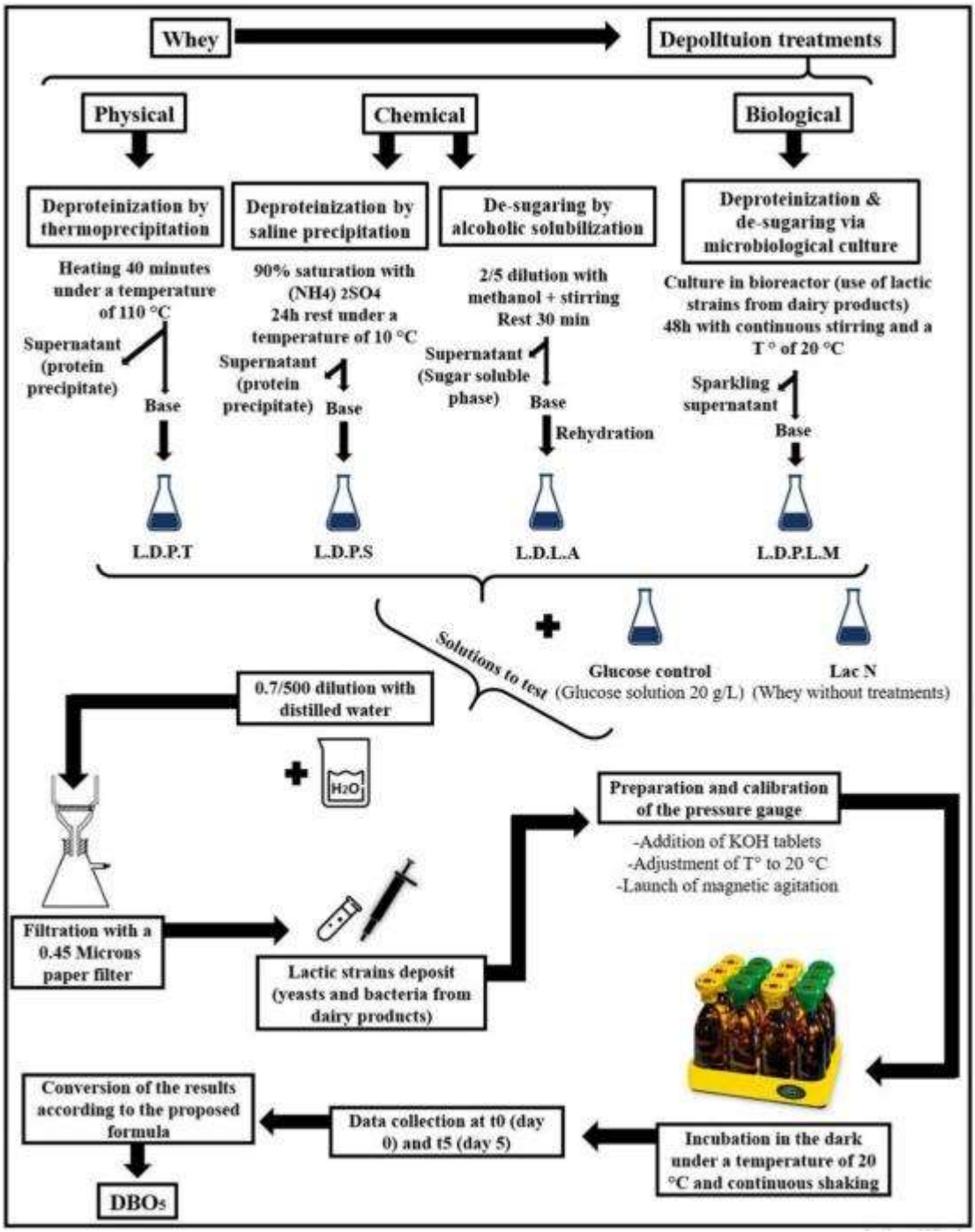


Fig.1 Summary of depollution protocols and their measurement.

Depollution tests

About the depollution tests, the results obtained are shown in **Figure 2**.

The Glucose Control had a BOD5 of 28 751 mg O_2/L which attests to the validity of the microbiological strains used for the measurement.

Lac N had a BOD5 of 50 000 mg O_2/L, a value that falls within the referential range proposed by Yorgun & al. 2008 (40 000 – 60 000 mg O_2/L).

The deproteinization by saline method L.D.P.S and by thermal method L.D.P.T had a depollution yield of 17% and 14% (BOD5 which reaches 41 428 and 42 857 mg O_2/L) respectively. This small difference is due to the fact that the salt used for saline

deproteinization was still present in the culture medium in small quantities and could have inhibited the growth of lactic strains in a rather weak manner.
De-sugaring by alcoholic method L.D.L.A allowed a rise in BOD5 which reached 70 000 mg O_2/L which represents a false positive explainable by two hypotheses:
-H1: The alcohol used for de-sugaring that remain present in the environment despite the dilution, had evaporated and interfered directly or indirectly with the measurement sensors integrated in the external caps of the opaque vials)
-H2: The alcohol present in the medium was so diluted that it could have been used as a source of carbon by microorganisms.
Deproteinization and de-sugaring by microbiological culture L.D.P.L.M had a depollution yield of 29% (BOD5 which nearly reached 35 000 mg O_2/L) which represents the best depollution yield. This result can be explained by the fact that the microorganisms used during microbiological culture had to degrade a maximum of nutrients (Proteins and Lactose) thus leading to a depletion of the medium in organic molecules and therefore of its polluting effect.
The L.D.P.L.M depollution yield can even reach 90% if carried out in better conditions as done by Gana.S & al., 2001 in their work on the DCO.

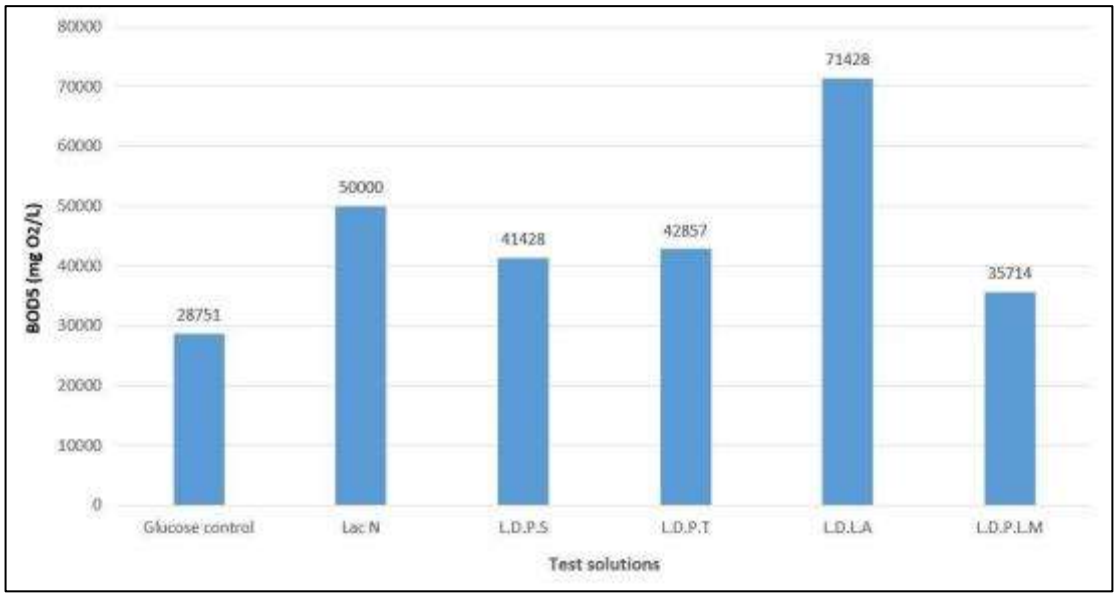


Fig.2 The variation in BOD5 according to the different treatments carried out on the harvested whey (as well as the controls).

*(**Glucose control** glucose solution at 20 g/L, **Lac N** whey without any treatment, **L.D.P.S** whey deproteinized by salts, **L.D.P.T** whey deproteinized by thermoprecipitation, **L.D.L.A** whey de-sugared by alcohol, **L.D.P.L.M** whey deproteinized and de-sugared by microorganisms).*

Extrapolation of the results

The extrapolation of the best results obtained to an industrial scale will be to opt for technologies such as ultrafiltration/nanofiltration or industrial bioreactors if we wish to totally depollute this waste and valorize it in an advanced way, or for drying/atomization in case we want to reduce the volume of the waste and recover it in its most practical form « powder » which is easily storable and salable (1 000 €/t in 2013, FranceAgriMer, 2013).

Conclusion

According to the tests carried out and the results obtained, deproteinization added to the desugaring of whey allowed a significant reduction of its BOD5: up tp 29%, thus enabling us to obtain a whey that can be rejected or reused as irrigation water without having harmful repercussions on the environment. There are several methods for depolluting whey: directly or through valorization processes. These methods are known and widely used in the West, and despite numerous researches and publications on the recovery and the polluting impact of whey in Algeria, industries that reject it, remain indifferent which is unfortunately, a common problem with several developing countries.

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AGRICULTURAL LAND AND HEAVY METALS

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Abstract

Intensive agricultural production is characterized by increased use of mineral fertilizers, pesticides, organic fertilizers and other substances to revitalize the soil or to increase the general condition of cultivated plants. The use of these substances increases the risk of accumulation, ie. increasing the concentration of heavy metals in the soil. Other factors, accelerated expansion of industrial plants, intensive transport and expansion of settlements also contribute to the introduction of heavy metals into the soil. Growing plants on land that has an increased concentration of heavy metals increases the risk of harmful substances entering the food chain. The paper presents the results of the analysis of 80 soil samples from 80 plots that were in the system of intensive agricultural production. No increased concentration of heavy metals was found in the analyzed soil samples. So far, in the area of Bosnia and Herzegovina, no symptoms have been seen on cultivated or wild plants due to the increased concentration of heavy metals. The plots from which the samples were taken were targeted because it was assumed that the results of the analysis would confirm the increased concentration of heavy metals. Arable land in Bosnia and Herzegovina is unpolluted and does not contain heavy metals, has a good structure and preserved natural fertility, which is why they are suitable for organic production.

Keywords: *heavy metals, land, intensive production, soil samples.*

Introduction

The concentration of heavy metals, in most agricultural land, is still not close to the MDK limit, but there is a constant fear that increased concentrations of heavy metals in the soil could affect human health. The fact that heavy metals cause numerous diseases, often fatal, has triggered numerous studies of the chemical behavior of heavy metals in soil and the mechanism of their uptake by cultivated plants.

Uncontrolled cultivation, fertilization, protection and implementation of other measures during the vegetation in order to achieve the highest possible yields, encouraged degradation processes in the soil that led to a decrease in fertility and soil quality. In addition to the decrease in the fertility of these soils, which is due to the decrease in the content of certain nutrients, there is a constant danger of accumulation of harmful and dangerous substances, which contributes to the growing deficit of soil moisture (Jelić et al., 2002; Živanović et al., 2002).

The origin of heavy metals in the soil is primarily geochemical, which means that they originate from the lithosphere and their concentration in the soil depends on the content in the rocks from which the parent substrate originated (Ubavić et al., 1993).

Heavy metals enter the food chain through plants, after which they show a cumulative effect in the human body, ie. they accumulate in certain organs or tissues where they exhibit harmful effects (Milošević and Vitorović, 1992).

Research has led to the simple conclusion that contaminated soils are the basic factor that conditions and contributes to the increased concentration of heavy metals in plant material. Plants grown on soil that is highly contaminated with heavy metals contain higher

concentrations of the same, compared to plants that grow or are grown on soil with a low content of the same elements.

The presence of heavy metals has been determined in natural phosphates, but also in other various mineral fertilizers, soil conditioners and organic fertilizers. It is especially important to point out that organic fertilizers, manure and compost contain higher concentrations of heavy metals. As a cause of constant use of organic fertilizers, there is an increased total concentration of heavy metals. Lands on which intensive vegetable production takes place are exposed to a significant risk of the appearance of larger quantities of heavy metals. This risk is justified because due to the lack of quality land, vegetable production is based on a limited number of plots with intensive cultivation and even increases the uncontrolled use of mineral and organic fertilizers, all accompanied by enhanced pesticide protection measures.

Pesticides, and especially fungicides, contain significant amounts of Cu, Zn, Fe, Mn and As, and some heavy metals are present in mineral fertilizers, the introduction of which contaminates the soil. Phosphorus fertilizers represent the greatest danger of heavy metals entering the soil, especially Cd, although the share of other heavy metals is significant. Concentrations of heavy metals vary significantly depending on the origin of the phosphate (Van Kauwenbeergh, 1997).

The consequences of contamination of agricultural land are manifested through phytotoxic effects and the negative impact of heavy metals on the quality of plant products. During the last decades, it is noticeable that with the development of industry, traffic, expansion of settlements and intensification of agriculture, various substances are used which cause increased concentration of heavy metals on agricultural land due to anthropogenic effects.

Recent researches confirm that the increasing presence of heavy metals in agricultural land is further endangered by inadequate application of chemicals, waste and cumulative water, sludge, as well as mineral fertilizers.

Fertilizer application, atmospheric sediment and plant leftovers can lead to increased cadmium (Cd) concentrations in the surface layer of the soil (Page et al., 1998). Atmospheric precipitation contributes to an increase in the amount of heavy metals in the soil, especially Pb and Zn, while Cr and V are predominantly of fertilizer origin, and fertilization and atmospheric precipitation have equal importance in soil contamination with As, Cd and Ni (Kadar and Ragalyi, 2010).

In long-term field experiments on chernozem (14-28 years), different fertilization systems were applied, which led to a significant increase in the content of some heavy metals (Cu, Ni, Pb, Cd) in relation to non-fertilized plots (Ubavić et al., 1995). Despite the significant increase in the concentration of these elements, the content of these elements was still below the permitted limit of heavy metal content in agricultural land. In thirty-year experiments with an increasing amount of phosphorus fertilizers, no significant increase in the concentration of Cd in the soil was found (Bogdanović et al., 1997).

In contrast, the results of field experiments in Rothamsted confirm that the application of fresh manure in quantities of 35 t / ha represents a more significant source of Cd for soil than Cd applied with phosphorus fertilizers and atmospheric sediment (Jones et al., 1987). The intensity of uptake and accumulation of heavy metals in plants depends on a number of factors and has not yet been fully elucidated. The pH value of the soil is one of the main factors determining the behavior and accessibility of heavy metals.

Material and Method

Land samples were taken from 80 plots on which the system of intensive vegetable production has been applied for a longer period (2012-2018 years). The research and sampling was conducted on the entire territory of Bosnia and Herzegovina. The plots that

were closer to industrial plants, settlements and traffic communications had an advantage when choosing for sampling because the starting point was the assumption that these plots would have an increased concentration of heavy metals. Soil samples were taken diagonally from several places on the plot from a depth of 0-30 cm. Soil samples were taken using a probe, and then a pooled sample weighing 1.5-2.0 kg was made from individual samples which was further used to perform chemical analyzes.

Results and Discussion

A significant number of elements that are most often associated with pollution and potential toxic effects can be classified in the group of heavy metals. The analyzes were aimed at determining the content of physiologically unnecessary, useless and toxic elements that belong to the group of heavy metals, namely: Cd, Cr, Hg and Pb, and semi-metals As, Ni, Cu, Zn are classified in the group of necessary microelements for growth and development of plants, but according to their physical and chemical properties they belong to heavy metals, which is why it was important to determine the content of these elements in the soil.

Table 1. Content of heavy metals in soil samples

	MAX.ALLOWED CONCENTRATION IN SOIL		Content-concentration		
ELEMENT (mg/kg)	INTEGRAL PRODUCTION	ORGANIC PRODUCTION	LOW	NORMAL	HIGH
nickel (Ni) 7.25-43.18	50	30	14	23	7
lead (Pb) 15.57-29.4	100	50	35	9	-
arsenic (As) 6.45-9.71	25	10	42	2	-
cadmium (Cd) 0.22-0.39	3	0.8	44	-	-
chrome (Cr) 23.23-37.02	100	50	41	3	-
mercury (Hg) <0,01-0.29	2	0.8	44	-	-
cooper (Cu) 16.49-37.38	100	50	40	4	-
zinc (Zn) 47.75-54.94	300	100	38	6	-

So far, in the area of Bosnia and Herzegovina, no symptoms have been seen on cultivated or wild plants due to the increased concentration of heavy metals. The plots from which the samples were taken were targeted because it was assumed that the results of the analysis would confirm the increased concentration of heavy metals. Producers confirmed that they used significantly higher amounts of fertilizers than recommended (organic, mineral, water-soluble, foil), that regular crop protection was carried out with fungicides and insecticides, and vegetables were grown with regular irrigation. The sampled land has a very good structure and fertility, which is why almost exclusively vegetable crops are grown on these lands, and crop rotation is not respected in most cases. This assumption is supported by the

fact that the mentioned plots are shifted, ie at least two, and most often three vegetable crops are grown during the year.

The results of the analysis shown in Table 1 refuted all assumptions because only in 7 samples a high concentration of nickel was found and ranged from 7.25 to 43.18, while the concentration of all other elements was extremely low or normal.

Extremely low concentration of lead was found in 88 samples, and the determined concentration in lead ranged from 15.57 to 29.4 mg/kg. The determined concentration of arsenic was also extremely low and ranged from 6.45 to 9.71 mg/kg. Two samples of arsenic, which were reported as samples with normal content, had values from 11.68 to 13.24 mg/kg. The cadmium concentration in all samples was very low, and ranged from 0.22 to 0.39 mg/kg, which is significantly below the maximum permissible concentration.

The table also shows the content of heavy metals in the soil, depending on the method, ie. production standards. The arsenic content in the soil is significantly lower than the limit concentration of arsenic for soils that are in the system of organic production. The chromium content was also very low with the content in the soil sample ranging from 23.23 to 37.02 mg/kg. A content of 52.14 was found in three samples; 57.14 and 58 mg/kg. The permitted mercury content in the soil in the system of organic production is 0.8 mg/kg, and in the analyzed samples the content ranged from <0.01 to 0.29 mg/kg. As with other elements, the mercury content was extremely low. The copper content is also very low, and these values ranged from 16.49 to 37.38 mg/kg, which is again below the standards prescribed for organic production. In crops that require copper for their normal growth and development, it is certainly necessary to provide this microelement during the vegetation. As with other elements, the zinc content is very low, but in relation to other elements it is closest to the limit value of 100 mg/kg which is the standard for organic production. The zinc content in the soil ranged from 47.75 to 54.94 mg/kg, but a significant number of samples had values ranging from 66.2 to 98.44 mg/kg.

Conclusion

The analysis of 80 soil samples did not reveal an increased content of hazardous substances in the form of heavy metals. Soil samples were taken from plots where intensive vegetable production has been taking place for many years, which contributed to the assumption that the analysis would determine increased concentrations of heavy metals.

The analyzes refuted all assumptions because the content of the analyzed elements was extremely low, ie. below the limit value of the content in lands that are in the system of organic production. Arable land in Bosnia and Herzegovina is unpolluted and does not contain heavy metals, has a good structure and preserved natural fertility, which is why they are suitable for organic production. The results of the conducted analyzes are encouraging because the presence of degradation processes has not been determined.

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THE LANDSCAPE AND VISUAL IMPACTS ASSESSMENT OF NORTH METN, LEBANON

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Abstract

North Metn is distinguished for its transformation throughout a short period of time, especially amongst its landscape elements, landscape characters and visual impacts from the coastal to its mountainous landscape. One methodology was proven to be efficient to this matter of study defining the Landscape and visual impact assessments LVIA "as a mechanism by which the landscape can be assessed against its capacity to accommodate change". Therefore, an assessment of the potential landscape and visual impacts of North Metn development was conducted in line with LVIA Guidelines. Its objective is to determine the existing landscape elements and visual quality and evaluate their impact. Thus, 82 viewpoints distributed from the coastal to the mountainous landscape were studied throughout this overpopulated casa of Lebanon, along with on-site observations, including site borders, primary roadways, significant valuable sites and public places. The study was divided into two parts. First, the Landscape Characters that were assessed by Landscape Quality, Landscape Value, Character Sensitivity, Landscape Visual Sensitivity and Magnitude and Significance of change. Second, the Visual Assessment which took into consideration the Visual receptor Sensitivity, the Magnitude and duration of change, the Visual intrusions and the change in the quality of the view compared to others. The results of both parts were therefore analyzed in order to classify the Significance of Visual Effects of the studied region as Major, Moderate, Moderate/Major, Minor or Moderate/Minor. Once the results were analyzed, North Metn's Significance of Visual Effects was ranked. In total, 65.85% of the district's landscape is between Major, Major/Moderate and Moderate ratings highlighting essential issues in the impact of change needed to be foreseen in the future landscape management.

Keywords: *Landscape character, Landscape and Visual impacts, North Metn, Significance and magnitude of change, Visual Assessment*.

Introduction

New studies have taken into form to understand the landscape modifications and impacts while suggesting new proposals and their impact on the surroundings. The Landscape and Visual impact Assessment methodology has proved to be an efficient way to study "the proposed schemes and developments that do not yet exist" (National Grid, 2013) It is known to be "a tool used to identify and assess the significance of and the effects of change resulting from development on both the landscape as an environmental resource in its own right and on people's views and visual amenity (National Grid, 2013). It has principle objectives of identifying and classifying the landscape likely to be affected by the construction and operation of the proposal and ancillary work, identifying the Visual Receptors with views of the proposed development and assessing the significance of effects on the prevailing landscape character and visual amenity, taking into account the measures proposed to mitigate

any impacts identified. (Guidelines of Landscape and Visual Impact Assessment 3rd Version, 2013)

However, in this study, the proposed approach will be thought of in a reverse way, judging by that the relative impacts of the existing constructions and land use. It took into consideration the Landscape Impacts in means of defining the "landscape as a resource in its own right" (Guidelines of Landscape and Visual Impact Assessment 3rd Version, 2013) and the Visual Impacts on "specific views and on the general visual amenity experienced by people". (Guidelines of Landscape and Visual Impact Assessment 3rd Version, 2013).

The choice of Site which is North Metn District is based on its geographical characteristics duration of development and transformation, heritage and land use. Thus, 82 viewpoints distributed from the coastal to the mountainous landscape were studied throughout this overpopulated casa of Lebanon, along with on-site observations, including site borders, primary roadways, significant valuable sites and public places. The criteria studied in details especially with different field visits and surveys are: landcover, landform, and landscape character types, Landscape Quality, Magnitude of Change, Receptors, Sensitivity, and Significance of change, Susceptibility, Visual amenity, geographical characters and zones of visibility. The results are a critical proof of the significant change in North Metn and its impact on the sensitivity and magnitude on the landscape itself, the visual landscape and the receptor.

The purpose of this research is to identify the existing landscape elements and visual quality within North Metn, and evaluate the impact on the landscape and the visual impact on the surrounding environment in the new proposal, new operation phase and construction process. The assessment addresses the main issues from the visual and landscape perspectives in order to arrive at mitigation measures to minimize the landscape and visual impact of the project. In the end, the size and scale of the effects will be proved within different maps, the geographical extent of the affected area and the duration of the effect and its reversibility (Redhurst Quarry ROMP – Volume 2A).

Materials and Methods

As mentioned before, the Landscape and Visual impact Assessment methodology is applied in reverse on the existing landscapes of North Metn and their significant magnitude of change and landscape and visual impacts. Landscape impacts including "direct impacts upon specific landscape elements within and adjacent to the site; and effects on the overall pattern of the landscape elements which give rise to the landscape character of the site and its surroundings; and impacts upon any special interests in and around the site." (Guidelines of Landscape and Visual Impact Assessment 3rd Version 2013) While the visual impacts include direct impacts of the development upon views in the landscape; and overall impact on visual amenity. (Guidelines of Landscape and Visual Impact Assessment 3rd Version 2013)

Therefore, a baseline study was considered necessary to "gain an objective understanding of the existing landscape and visual conditions within the study area" (South kesteven district council development services 2018). Based on this study, 82 viewpoints throughout the North Metn landscape were chosen based on their contour lines, geology, land use and the soil type.

They were chosen in a way to even spread the visual envelope of the Site: Point considering a human's normal field of vision and panoramic view, locations which represent a range of near, middle and long distance views, views from both public and private viewpoints and views from sensitive receptors within designated landscapes. (Landscape and Visual Impact Assessment Methodology Rev A 2012)

They were distributed from the coastal to the mountainous landscape throughout this overpopulated district. Each viewpoint had a unit name, northing and easting, direction of view, altitude and distance from viewpoint.
Then, field visits around the site were considered for an on-site observations, including site borders, primary roadways, secondary roads, highways significant valuable sites and public places. The roads of study were chosen based on their geographical criteria, everyday use, ratio of passengers and users and their cohesion with the landscapes.
The approach was applied to identify the effects on the landscape resource and Visual Aminity as experienced by people. (South kesteven district council development services 2018) Therefore, the Landscape Character assessment and the Landscape Visual assessment took place: At each viewpoint on the field, the tables below were filled taking into consideration: the Receptor Sensitivity, the Magnitude of Visual Impact, The Significance of Visual Effects, the Landscape Character Assessment, the Magnitude of Landscape Impacts and the significance of Landscape effects.

LANDSCAPE VISUAL ASSESSMENT

	High	Medium	Low
RECEPTOR SENSITIVITY			

MAGNITUDE OF VISUAL IMPACT

	Very Large	Large	Medium	Small	Very Small
MAGNITUDE					

SIGNIFICANCE OF VISUAL EFFECTS

	SENSITIVITY		
MAGNITUDE	High	Medium	Low
Very Large			
Large			
Medium			
Very Small			
Negligible			

Figure 1: Landscape Visual Assessment (LVA)

LANDSCAPE CHARACTER ASSESSMENT

	Very High	High	Medium	Low	Very low
LANDSCAPE QUALITY					
LANDSCAPE VALUE					
CHARACTER SENSITIVITY					
LANDSCAPE VISUAL SENSITIVITY					

MAGNITUDE OF LANDSCAPE IMPACTS

	Large	Medium	Small	Negligible
MAGNITUDE				

SIGNIFICANE OF LANDSCAPE EFFECTS

	SENSITIVITY			
MAGNITUDE	Very High	High	Medium	Low
Large				
Medium				
Small				
Negligible				

Figure 2: Landscape Character Assessment (LCA)

Thus, an evaluation of the landscape and visual sensitivity of the receptors based on the susceptibility of the receptor to the type of change occurred. The magnitude of the effect and whether it is reversible or not. Both the Sensitivity and magnitude evaluation are combined to determine the "overall effect" which itself is evaluated and studied to a wide number of factors such as direction of view and distance of the viewpoint. (South kesteven district council development services, 2018)
The results are studied along the district, taking into consideration its roads, soil type, geology, land use and contour lines. First the Landscape Character Assessment (LCA) results are mapped separately to all the mentioned criteria then the landscape Visual Assessment (LVA) results are also mapped. Both are analyzed for each criteria by itself and then analyzed as a combination to understand the relation between the ratings and the characters of the North Metn district.

Results and discussion

North Metn's contour lines shows its variety of altitude, going all the way from 50 m to 2600 m above sea level. By that it is known for a wide variety of land use from agricultural, vegetative, natural, constructed lands. Besides, the roads are essential to study in order to understand the receptor point of view and his sensitivity towards the change. In figures 3 & 4, we see the results of LVA and LCA related to the roads of North Metn

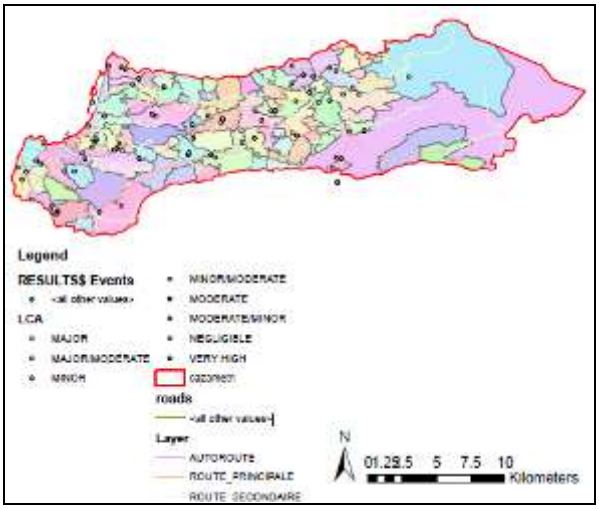


Figure 3: LCA results on Roads of North Metn

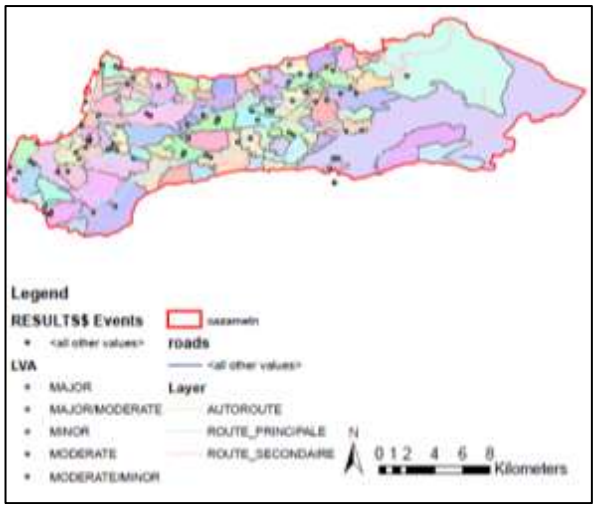


Figure 4: LVA results on Roads of North Metn

Based on the figures above, the relation between the roads and the results is clear that it will have different impacts on the receptor and on the landscape as seen in the table below. It is clear that each time we go higher by altitude, the changes are less so is there impact which is nearly negligible to moderate, however, the visual impacts on landscape and its characters are very high to major on the receptor and its surroundings. Highways have the most impact on receptors and landscape because of their construction as a result of landscape destruction.

Table 6: LCA & LVA results on contour lines and Roads of North Metn

	LCA								LVA				
CONTOUR LINES	very high	major	major/ moderate	minor	minor/ moderate	moderate	moderate/ minor	negligible	Major	Major/ Moderate	Minor	Moderate	Moderate/ Minor
0-600 m													
600-1500 m													
1500 - 2600 m													
ROADS													
Highway													
Primary roads													
Secondary roads													

Then, The LCA and LVA results were studied thoroughly in a relation with the contour lines leading to the different altitudes and the land use. Here, in the figures below, it is clear the distribution of the LCA & LVA results along the land use and contour lines of North Metn.

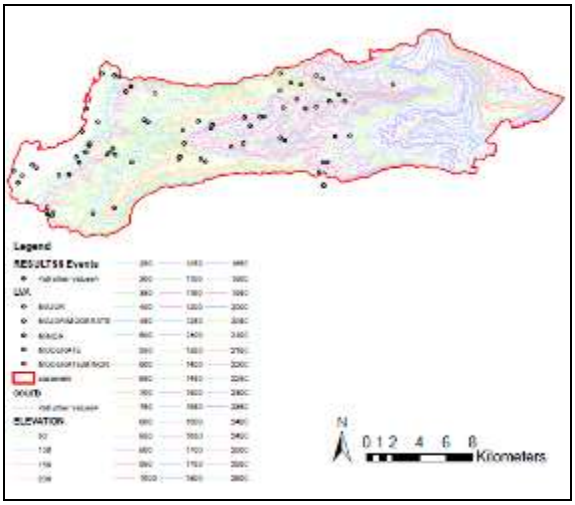


Figure 5:LVA results on contour lines

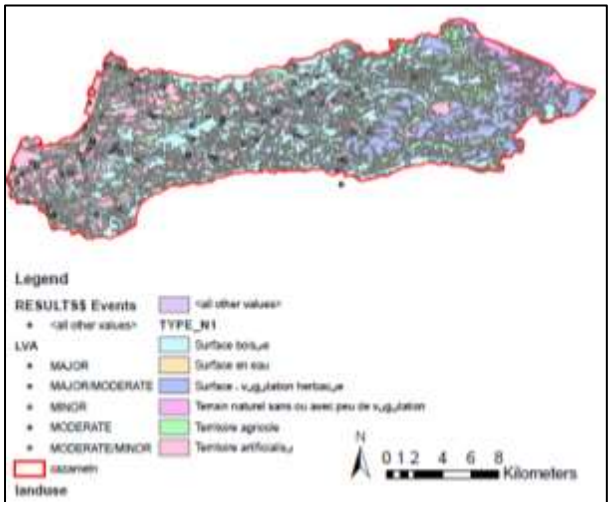


Figure 6: LVA results on land use

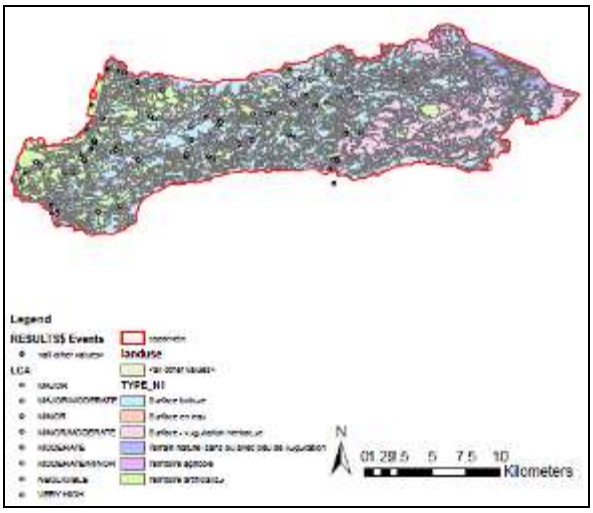


Figure 7: LCA results on land use

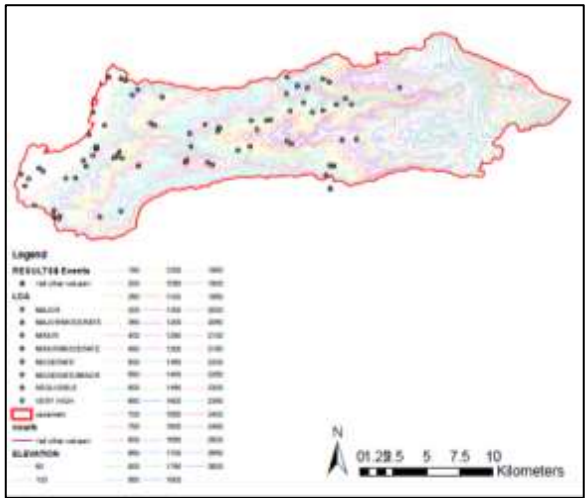


Figure 8: LCA results on contour lines

Moreover, the results were combined to compare them and their significance of change to try and understand how the impact and magnitude of change is related to the altitude and land use occupied by the human activities. Thus, the table below shows that the higher the altitude, the lower is the change while the higher the altitude the higher is the receptor sensitivity because of the natural and green sceneries that attract the receptor.

Table 7: LCA & LVA results related to landuse and contour lines

		LCA								LVA				
CONTOUR LINES	LANDUSE	very high	major	major/ moderate	minor	minor/ moderate	moderate	moderate/ minor	negligible	Major	Major/ Moderate	Minor	Moderate	Moderate/ Minor
0-600 m	wooded area													
	artificial area													
600-1500 m	wooded area													
	vegetation area													
	agricultural area													
	water area													
1500 - 2600 m	water area													
	vegetation area													
	natural area or with few vegetations													
	agricultural area													

In the end, the significance of the visual effects is determined by the assessment of receptor sensitivity set against the magnitude of change. As for 'Significant' landscape effects would be those effects assessed to be severe, major or major/moderate (Landscape and Visual Impact Assessment Methodology Rev A 2012) North Metn's Significance of Change was ranked. In total, 65.85% of the district's landscape is between Major, Major/Moderate and Moderate ratings highlighting essential issues in the impact of change needed to be foreseen in the future landscape management in the area.

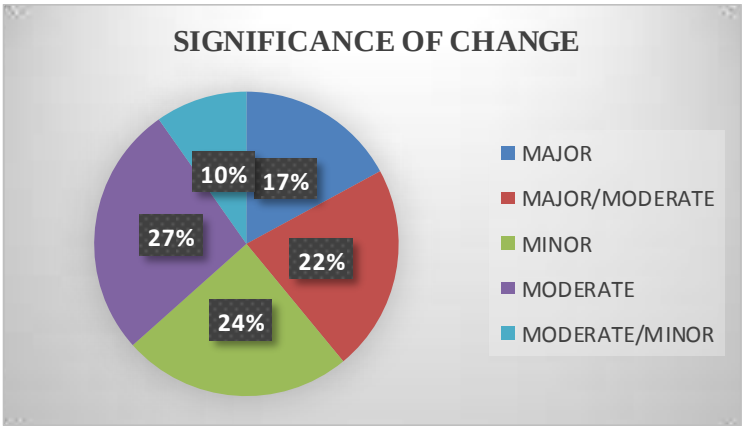


Figure 9: Significance of change

Conclusion

The Landscape Visual Impact Assessment follows accepted standard procedure. It assesses the ways in which the proposed development would have an effect on the existing landscape character and visual amenity of receptors in the surrounding area. This included a baseline study of the existing site and its surroundings, a study of the landscape and visual (Landscape and Visual Assessment Methodology Apendix 6.1) By considering the criteria and the land use of the certain landscape studied, a lot could be concluded concerning the visual receptor and the landscape itself and the relation between them that is affected by change. This shows the importance of the magnitude and sensitivity of change criteria. Some usually consider this method to study the effect of a proposed project to be implemented to a landscape,

However, the reverse way that this study used showed the impact of change to the landscape that North Metn has and the impact of these changes to its visual receptors. Such factors considered may include for example, the potential for weather conditions to restrict views, the principle aspect of the viewpoint/viewer, the proportion of any particular view affected, the potential for the development to attract the eye or to become a focal point in the view to the detraction/benefit of competing visual elements, etc. (Corscaden Associates, 2019)

Once sensitivity to change and magnitude of impact have been classified, the two are considered to produce an assessment of the significance of effect experienced by each visual receptor. Significance of effect is not absolute and can only be defined in relation to the location of receptor and nature of development. It should be noted that visual effects may be either adverse (negative) or beneficial (positive) in nature. (Corscaden Associates, 2019) As seen already by the results, sometime the Major impact on the receptor caused by the change can be Major but it has to be analyzed to its location and users in order to understand if it is a negative or positive impact.

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ENHANCING ROAD SAFETY THROUGH ROAD LANDSCAPE DESIGN THE CASE OF METN EL SARIH HIGHWAY- LEBANON

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Abstract

Lebanon is the 86th country in car crashes worldwide according to the World Health Organization (2017) and the Service of Technical Studies for Roads and Highways showed that 74% of the driver's failure comes from wrong information sampling and treatment leading to road accidents. Knowing that the road is not independent of its surroundings and that it should manipulate the legibility and the visibility for the user to control his behavior and speed, a study was conducted along Metn El Sarih highway (12 km) because of its road landscape conditions related to the highest car crash rate and low level of safety in the North Metn's casa, going from its urban cities to the mountainous areas. Therefore, surveys were conducted in order to understand the users' perception of the road, their legibility to its safety and level of visibility and access. Besides, aerial maps from different areas of the surrounding municipalities were studied along the way. As a result, 5 spots were chosen for a road safety intervention because of their bad visibility, inconvenient road conditions and dangerous turns. Thus, the cuts and fills of these spots were considered to study the angle of visibility and its weaknesses for a suitable intervention. The results were implemented on detailed maps, sections and sketches to demonstrate the road landscape safety design, accompanied with comparative studies of the safety's value, by adding the convenient greenery depending on its height, texture, shape and color, applying urban furniture and amenities in seated public spaces as well as observatories with parking spots facing landscape views serving as distractions. This road landscape design increased the visibility and legibility required to reach safe road landscape standards.

Keywords: *Landscape, Road Landscape, Road Safety, Road Landscape Design, Lebanon.*

Introduction

Motor vehicle crashes are the ninth leading cause of death worldwide and anticipated to become the seventh by year 2030 (Shackford et al., 1993; World Health Organization, 2013). Safe driving relies on vision (Owsley and McGwin Jr 2010), however, Visual illusions, predispose healthy people to recurrent mistakes when judging size, position, and motion (Bressan et al., 2003; Horswill et al., 2005). There is reason to believe that the landscape along roads affects driver's behavior, which might influence traffic safety as well (Gibson and Crooks, 1938; Drottenborg, 2002; Sétra, 2006). The road should not be considered as being independent of the environment it passes through, the road improvement strategy has much in common with the overall project design including the road and the surrounding land and operates at route level, with the addition of a dynamic analysis (Sétra, 2006). The collaboration between roads and landscape can enhance the safety on the roads within the landscape design. The landscape in which the road is present has a huge effect, whether on the driver, the driver's behavior, and other passengers in the cars or even the pedestrians.

Knowledge of how drivers react to their surroundings and to what they react has left few significant traces in governing documents. Several general recommendations can be found, however, including that the spatial form of a road or street should give clear visual guidance and signals to drivers regarding suitable speed and driving behavior (VGU, 2012). The environment interpretation influences the driver's behavior. As wrong interpretation of the road elements may cause accidents, so modifying the road environment should improve its legibility. The landscaped improvements bring a long-term action. On its own, landscape is not sufficient to get rid of all the accidents but it contributes generously to road safety.
The road landscape design is defined by a system that shows how human, environment and vehicle react, it also shows its effect on the road landscape, and all road elements. Additionally, this study highlights the road legibility which describes how every person interprets road or landscape signals differently, leading to the importance of the user's perception. Lebanon is the 84th country in car crashes worldwide according to the World Health Organization (WHO). WHO also revealed that road traffic accidents were the 5th cause of death in Lebanon (World Health Organization, 2018). This study was conducted along Metn El Sarih highway (12 km) because of its road landscape conditions related to the highest car crash rate and low level of safety in the North Metn's casa, going from its urban cities to the mountainous areas. The objective of this paper is to analyze the importance of road legibility and visibility, along with road elements, and its implementation in order to increase safety through the road landscape design.

Materials and Methods

This paper is based on extended data collection and research that started in 2016 and continued to check potential evolution, in order to further understand the connection between road and landscape. Road landscape studies that tackle road elements, their function, value and implementation were analyzed. The Service of Technical Studies for Roads and Highways (Sétra 2006) revealed that 74% of the driver's failure comes from wrong information sampling and treatment leading to road accidents. To further understand the link between road visibility, legibility and user's perception, a breakdown of the mental activities linked to driving is shown in the diagram below:

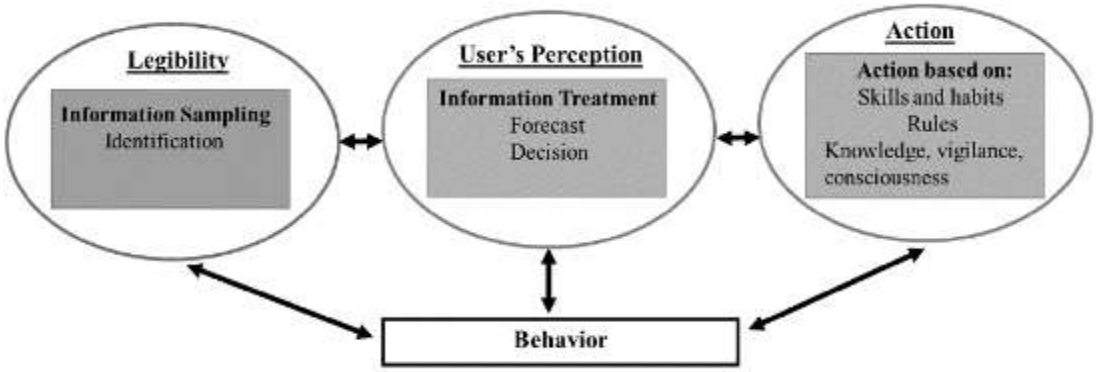


Fig 1: The mental activities related to driving

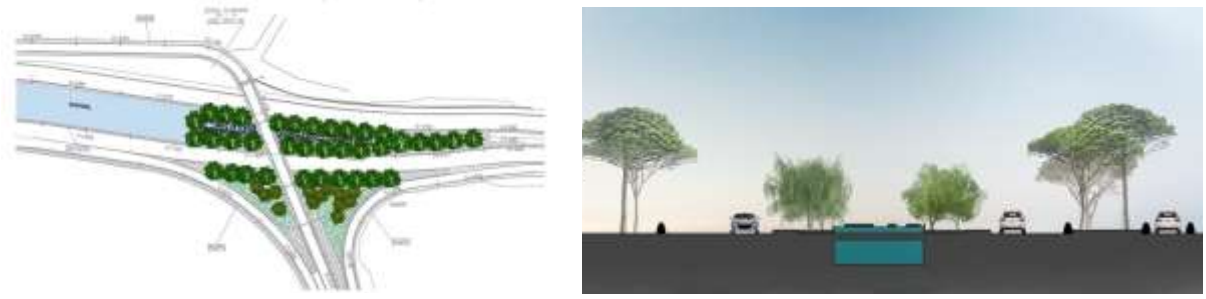
Additional data was collected during 3 months by a face to face questionnaire carried out on 100 participants between the ages of 19 and 55 of all genders, divided into people that take this road as driver's and as passengers. Apart from data about the participants (name, age, occupation, driver's license) different issues regarding the safety of the chosen site were addressed: Speed limit, aesthetics of the road, safety measures, necessity to take this road, substituting this road to reach destination, number of accidents that occur on this road.

The Metn Sarih highway was profoundly studied all through, starting with its evolution through time along with its surrounding networks. The soil occupation domain, surfaces and networks, the mesology domain (air, noise, water and soil pollution). Site visits conducted to assess the situation of the road landscape, pictures every 400m along the 12km were taken from a driver's perception and from a passenger's perception to help put together a table of comparison showing the weak and strength locations on site accompanied by sections through google earth that helped assess where the road was excavated or filled. This analysis disclosed 5 major areas where safety is lacking and where an intervention using the landscape can increase road safety. Those 5 intervention areas have higher risk of accidents due to distractions by surrounding excavation sites of large scale effecting the driver's perception, directional confusion by user's due to lack of directional signage and numerous interlocking connections, heavy maneuvering turns without any alarming signage that require a strict abidance to speed limit while driving and eventually lack of optical guidance for user's. Programs were used to analyze the data collected and to propose results such as google maps, AutoCad, Rhino, and Photoshop.

Results and Discussion

The survey conducted revealed that 73% of the participants find the road unsafe and avoid taking it unless it is necessary whereas 5% find it extremely dangerous. It also revealed that most participants don't notice the site's aesthetic value since they exceed the speed limit considering this highway allows them to reach their destination faster. The data collected demonstrated that a project's assessment should consider the formation of the road (median strips, intersections, roundabouts), levels of service (number of lanes), design speeds of the major road/s (all minor roads leading into junctions), traffic volumes (average annual daily traffic), road geometry, road alignment (curve radius, including sight triangles at intersection/conflict points), line markings, traffic signalization and operational signage and safety barriers. The design process to implement safety should respect the clear zone, sight distance, clearance to other elements, pedestrian and cyclist safety, functional planting for safety, and maintenance (Queensland Government 2013). The analysis of the Metn El Sarih Highway data disclosed the spots at highest risk of accidents and lowest safety measures. The interventions seeking to improve safety conditions were treated based on the concept of creating optical illusion to serve as optical guidance, such as the use of endemic plants to integrate an area in its surrounding site, whereas non-endemic plants were introduced in certain areas with the purpose of attract user's attention to send out a certain message (alarm the user's of a dangerous turn, lower speed).

First intervention area:



(a) (b)

Fig 2: First intervention area: Metn El Sarih Entrance. (a): Plan (b): Section

To serve the safety purpose, optical guidance was created with an alignment of endemic trees. The Channel of water that is not visible to driver's was recalled by a water mirror with stepping stones surrounded by a green public space for pedestrians.

Second intervention area:



(a) (b)

Fig 3: Second intervention area: Divergent Median. (a): Plan (b): Section

This median on the highway is surrounded by crushers and excavation sites on both sides. Straight ahead a high mountain excavated creating a distraction for the driver effecting the safety on the road. For that reason, a divergence was created by introducing non-endemic trees with unfamiliar colors in the region attracting the attention of the driver keeping his focus on the median thus on the road itself leading to a decrease in the numbers of accidents, along with diverting the driver's attention from the unaesthetic view.

Third intervention area:



(a) (b)

Fig 4: Third intervention area: Metn El Sarih Rest Area. (a): Plan (b): Section

This turn is the most dangerous one on this highway. To alert the driver, an introduction of non-endemic trees and shrubs of an alarming color, red, is implemented 100m earlier in a shy matter to signal a turn, and the signal strengthens the closer we get to the heavy turn. A ramification that leads to a different village was used to create a rest area since by code, heavy vehicle drivers should stop at a rest area every 2h. It has a sustainable thirsty concrete parking for regular and heavy vehicles, a place to rest securely and enjoy the view of the water channel passing all at once.

Forth intervention area:



(a) (b)

Fig 5: Forth intervention area: Metn El Sarih Observatory. (a): Plan (b): Section

This turn is the second most dangerous along this site, heavy maneuvering with a hard cliff on the other side, any driver who exceeds the speed limit is at high risk to be thrown off track. A punctual introduction of non-endemic trees and shrubs with the alarming color red. With this cliff comes an astounding view causing people to slow down without prior warning to enjoy it. Creating the observatory helps reduce these accidents and adds function to this space. A pedestrian lane and a bike lane are created as promenade to reach the observatory. The functions are separated from the road with a ditch and stiff shrubs that resist collision.

Fifth intervention area:



(a) (b)

Fig 6: Fifth intervention area: Atchane Ramification /Beit Misk Entrance. (a): Plan (b): Section

This section of the highway is very confusing due to 6 entrances and exists. In an attempt to create an optical guidance, endemic familiar trees are planted on the roads that lead from Beirut to Baabdet and the opposite, whereas the non-endemic trees with a soothing yet unfamiliar color indicating the roads that lead to Atchane ramification. The optical guidance decreases the confusion while driving thus increases road safety.

Conclusion

The evolution of spaces and increasing addition of roads by the minute creates a necessity to reflect on the undesirable consequences that comes with, and aiming to integrate these roads withing their surrounding with the least impact possible. Concerns on the subject of safety are raised with the intention to diminish casualties. However, high amenity outcomes can be achieved as much as visually integrating structural components within the surrounding landscape, as much as they can by highlighting the element as a feature element. In most cases the highest road landscape amenity value can be achieved by rendering the component as visually recessive as possible, such that its visual impact effects lightly on the surrounding landscape. Urban design treatments should aim to support and promote the safety function of civil design by addressing safety in design for construction, inspection, maintenance. It should promote the legibility of the road landscape for road users, minimizing risk of road user distraction (limiting unnecessary visual intrusion), promote visually recessive treatments and address risk of light reflection (Queensland Government 2013).

This Article's objective was to develop awareness on road safety, emphasis on the importance of the integration of landscape by creating optical illusion in order to increase road safety through the road landscape design.

Applying this strategy on constructed roads would be challenging in terms of the possibility of implementing newly introduced elements.

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IS THE EXPLOITABLE BIOMASS (B) VULNERABLE TO CHANGES OF NATURAL MORTALITY (M) BY AGE? CASE OF THE ROUND SARDINELLA

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Abstract

A proper estimation of biomass is critical for environmental and decision making to conserve fish stocks. This work aims to explore the vulnerability of biomass estimation to the variability of natural mortality M by age. 2460 individuals of all sexes, sizes ranging from 7.25 to 21.75 cm, were studied. Monthly sampling was conducted from December 2018 to March 2020 from landings of seiners in the central region of Algeria between Tenes and Bejaïa. Descriptive statistics were performed using FISAT II and VIT4Win. The results generated two different scenarios: first, the current total biomass (Bc) was estimated at 8686.6 tons for M = constant = 0.51 yr^{-1}; secondly total biomass was estimated at Bc = 10636.4 tons for M = variable by age n, M_n, [M_1 = 1.130 ; M_2 = 0.791 ; M_3 = 0.721 ; M_4 = 0.606 ; M_5 = 0.545 yr^{-1}]. Evaluating Bc with constant M showed a decrease of 1914.19 tons compared to biomass assessment with Mn. Judging by these results, considering that mortality M varies with age has a great impact on biomass evaluation. Naturally, juvenile fish are exposed to larger predation mortality than an adult fish. This difference in predation mortality may be quite significant. Finally, we recommend adjusting the catch effort factor F_C = 1 to $F_{0.1}$ = 0.54 for M variable. This precautionary approach would permit long-term renewal of the stock of *S. aurita* in the central region of the Algerian coast (increasing sea biomass from 10632.91 to 14848.9 tons).

Keywords: *Sardinella aurita, Algeria, Biomass (B), Mortality (M), Stock assessment.*

Introduction

Fishing in Algeria constitutes an economic activity that provide not only a food source for the population but also thousands of jobs for the Algerians. Fishing has to be controlled and handled according to a sustainable approach to maintain this resource. Thus, fish stocks biomass needs to be constantly evaluated while making sure that the values obtained are the most representative of the reality. In literature many authors as Benamar (2011), Amponsah (2013) and many others, conducted fish stock biomass assessment with the assumption that natural mortality (M) is a constant value (M_{cte}) that isn't linked to fish age. However, it seems that fish mortality (M) varies with age according to Garrido (2016) and Quattrocchi and Maynou (2017), its value is the highest at the youngest age and diminishes with the aging of the fish This can be explained by juveniles, eggs and fish larvae being the most prone to predation and less resistant to environmental conditions than adult fish. This work aims to underline the impact of natural mortality values used when assessing fish stock biomass, in the case of *Sardinella aurita* (Valenciennes, 1847), a small pelagic fish of the family of clupeoids. To do so, we compared the biomass assessed while considering natural mortality as a constant (M_{cte}) with the biomass calculated using natural mortality as a variable (M_n). This study was conducted in the central region of the Algerian coast between Tenes and Bejaïa.

Material and Methods

Monthly sampling was conducted from December 2018 to March 2020 from landings of seiners situated in the central region of the Algerian coast, 2460 *Sardinella aurita* individuals of all sexes were sampled and each eviscerated and weighed (W_{ev}) and measured from the extremity of the fish muzzle to the end of the caudal fin (TL). Sizes were arranged into length classes ranging from 7.25 to 21.75 cm, with a class width of 0.5 cm. The age-length key was then calculated using Bhattacharya's (1967) method following the recommendation of the DYNPOP working group of the CIESM (Abella *et al.*, 1995; Aldebert and Recasen, 1995; Alemany and Oliver, 1995 and Campana, 2001 in Bouaziz, 2007). The estimation of the age-length key using Bhattacharya's method was performed on the software FISAT II version 1.2.2 (Gayanilo and Sparre, 2005). We then used the software VONBIT_ALXL (2012) to assess the von Bertalanffy's (1938) growth parameters (L_∞, K and t_0), which is a fish growth model translating into the equation: $L_t = L_\infty(1 - e^{-K(t-to)})$.

We then used the growth parameters obtained to calculate the mortality of the fish stock (M_{cte}) using Djabali's *et al.*, (1993) natural mortality equation:

$$\text{Log}_{10} M = -0{,}0278 - 0{,}1172\ \text{Log}_{10} L_\infty + 0{,}5092\ \text{Log}_{10} K$$

To calculate mortality for each age ($M_n, n = \text{age}$), we first calculated the K for each age using von Bertalanffy's model equation as follows:

$$L_t = L_\infty\left(1 - e^{-K(t-t_0)}\right)$$
$$L_t = L_\infty - L_\infty e^{-K(t-t_0)}$$
$$(L_t - L_\infty) = -L_\infty e^{-K(t-t_0)}$$
$$\ln(L_\infty - L_t) = \ln\left(L_\infty e^{-K(t-t_0)}\right)$$
$$\ln(L_\infty - L_t) = \ln L_\infty + \ln e^{-K(t-t_0)}$$
$$\ln(L_\infty - L_t) - \ln L_\infty = -K(t - t_0)$$
$$K = \frac{\ln L_\infty - \ln(L_\infty - L_t)}{t - t_0}$$
$$K = \frac{-\ln\left(\frac{L_\infty - L_t}{L_\infty}\right)}{t - t_0}$$

We calculated L_∞ for each age ($L_{\infty n}$) using Taylor's (1962) in Bouaziz (2007) equation:

$$L_\infty = \frac{L_{max}}{0{,}95}$$

And then we used Djabali's *et al.*, (1993) natural mortality equation cited above to calculate mortality for each age (M_n) as the following:

$$\text{Log}_{10} M_n = -0{,}0278 - 0{,}1172\ \text{Log}_{10} L_{\infty n} + 0{,}5092\ \text{Log}_{10} K_n$$

To calculate fishing mortality (F) we first calculated total instantaneous mortality rate (Z) using FISAT II, and then we calculated fishing mortality (F) using the following equation:

Knowing Z=F+M therefore F=Z-M

We also calculated the length weight relationship ($W_t = a\ Lt^b$) constants a and b.

After having calculated VIT4Win inputs, that said, mortality F, M_n, M_{cte} and the von Bertalanffy's (1938) growth parameters (L_∞, K and t_0), we ran the software VIT4win 1.3 (Lleonart and Salat, 2011) to assess current (Bc) biomass using M_n and M_{Cte}.

Results and Discussion

The estimation of the average length by age resulted of the following:

Table 8. The average length by age for the population of Sardinella aurita sampled using FISAT II.

Age (year)	Mean length (cm) ± Standard deviation	Population (NI)	Population %	Separation index (S.I.)
1	10.59 ± 0.960	889.75	36.20%	n.a
2	14.44 ± 1.290	1309.76	53.28%	2.280
3	17.25 ± 0.510	186.28	7.58%	2.130
4	19.11 ± 0.370	68.04	2.77%	2.110
5	22.75 ± 0.420	4.34	0.18%	2.290

Data presented for mean length and SD.
S.I. > 2 at all ages, therefore, the separation index is significant. This means that, the decomposition of size frequencies of our sample reflects representative results.

Bhattacharya's method performed on the software FISAT II 1.2.2 provided us with the decomposition of our sample into five (05) cohorts with a median length of 10.59, 14.44, 17.25, 19.11 and 22.75 cm for the ages 1, 2, 3, 4 and 5 respectively.
According to the table 1, age 1 and 2 are the most present in our sample, constituting 36.2% and 53.3% respectively. On the other hand, cohorts of age 3, 4 and 5 constitutes only around 10% of our sample, therefore it is likely that ages 1 and 2 are the most caught by seiners in the study zone.

The calculation of L_∞ and K (growth parameters of the whole sample):
By entering the age-length values to the VONBIT_ALXL (2012) we obtained the following growth parameters, L_∞ = 53.16 cm, K = 0.08 year^{-1}, t_0 = -1.838 year, which we consider as aberrant. This can be explained by L_∞ not being close to the value of L_{max} (the maximal length of the sample, which is 22.8 cm) as it should according to Pauly and Moreau (1997) and Bocar *et al.,* (2019), also K in this case has a low value that does not represent the growth speed of small pelagic fish which has a fast growth (Pauly et Moreau, 1997 and Bocar *et al.,* 2019), as for t_0 the value obtained doesn't make sense regarding this specie firstly because the later has a low longevity (05 years in this case), moreover, this value of t_0 seemed aberrant when compared to the values of t_0 obtained in other studies (Tsikliras et *al.,* 2005; Bouaziz, 2007; Apostolidis and Stergiou, 2014; Benamar, 2019), these results can be the effect of a non-regular growth of the fish caused by ecosystemic disturbances, this non-regular growth would then translate into an irregular growth curve (in other words a growth graphic with non-distinguishable curvature), which in turn distort the growth values obtained. To correct the growth parameters obtained we added the values age = 0 and TL = 0 to the age-length key, when using VONBIT_ALXL (2012), doing so, we considerately improved the curvature of the growth graphic, as a result, we obtained growth parameters that are largely more representative of the specie in reality, that are: L_∞ = 23.54 cm, a value close to L_{max} = 22.8 cm, K = 0.48 year^{-1} a value representative of the fast growth of small pelagic fish, t_0 = -0.043 year a t_0 that concords with literature (Tsikliras et *al.,* 2005; Bouaziz, 2007; Apostolidis and Stergiou, 2014; Benamar, 2019).

The calculation of asymptotic length ($\mathbf{L_{\infty}}$) for each age ($\mathbf{L_{\infty n}}$, asymptotic length for each age n) using Taylor (1962) in Bouaziz (2007) equation and K_n using von Bertalanffy's model equation provided us with the following results:

Table 9. Values of $L_{\infty n}$ (asymptotic length) and K_n (curvature parameter of the von Bertalanffy growth function) calculated for each age.

Age classes	$L_{\infty n}$ (cm)	K_n (year^{-1})
1	12.11	1.99
2	16.32	1.06
3	18.42	0.91
4	20.53	0.66
5	24.21	0.57

Results of the calculation of the mortality $\mathbf{M_{cte}}$ and $\mathbf{M_n}$ using Djabali's *et al.*, (1993) natural mortality equation with K_n and $L_{\infty n}$ results for each age (table 2):

$$M_{cte} = 0.51 \text{ year}^{-1}$$

$$M_1 = 1.13,\ M_2 = 0.79,\ M_3 = 0.72,\ M_4 = 0.61,\ M_5 = 0.54$$

The results obtained show that mortality varies with age, it is the highest at age 1 and then diminishes with age (rapidly at first and then diminishes with a slower pace) which concords with Garrido (2016) and Quattrocchi and Maynou (2017).
Concerning length weight relationship ($W_t = a\ Lt^b$) constants a and b, we obtained the following:

$$a = 0.0045 \text{ and } b = 3.123$$

The results of biomass assessment using VIT4Win 1.3:
Critical age and size of the virgin stock (mean size and age of the cohort having the highest biomass) with M_n were 2.20 year, 15.5 cm meanwhile with M_{cte} were 2.63 year, 17 cm. Opposingly mean age and size of the current stock didn't shift much (1.37 year, 11,21 cm for M_n and 1.43 year, 11.54 cm for M_{cte}). The same conclusion was drawn for the current stock critical age and length which were 1.63 year, 13 cm for M_n and 1.73 year, 13.5 cm for M_{cte}.
Concerning exploitable biomass, we used the virtual population analysis (VPA) feature in VIT4Win software to obtain the results presented in the table 3. Using natural mortality as a constant (M_{Cte}) the current biomass obtained was Bc = 86866.6 tons (biomass Bc = (Bc/R)*R, R is the number of recruits). On the other hand, when assessing biomass using mortality as a variable (M_n) we obtained Bc = 10636.4 tons, a value different from Bc calculated with M_{cte} by 1949.8 tons.

Table 10. Yield and biomass parameters obtained by the VIT4Win 1.3, for round sardinella, *Sardinella aurita*, of the central region of the Algerian coast.

Parameters for M_{Cte}					Parameters for M_n				
	Factor	Y/R (g)	B/R (g)	SSB/R (g)		Factor	Y/R (g)	B/R (g)	SSB/R (g)
F(0)	0	0	41.024	34.093	F(0)	0	0	20.929	15.967
F(0.1)	0.400	9.202	18.918	12.681	F(0.1)	0.540	5.176	10.152	5.657
F_{MSY}	0.700	9.808	13.205	7.394	F_{MSY}	1.200	5.664	6.521	2.485
F_C	1	9.585	10.092	4.675	F_C	1	5.640	7.272	3.103
Number of recruits: 860736484.88					Number of recruits: 1462652863.53				

Factor = Factor of effort, Y/R = Yield per recruit, B/R = Biomass per recruit, SSB/R = Spawning stock biomass per recruit, F(0) = Factor of non-fishing, F(0.1) = factor of fishing mortality rate at which the marginal yield-per-recruit is only 10 percent of the marginal yield per recruit on the unexploited stock, F_{MSY} = factor of fishing mortality rate corresponding to the maximum sustainable yield, F_c = factor of current effort fishing.

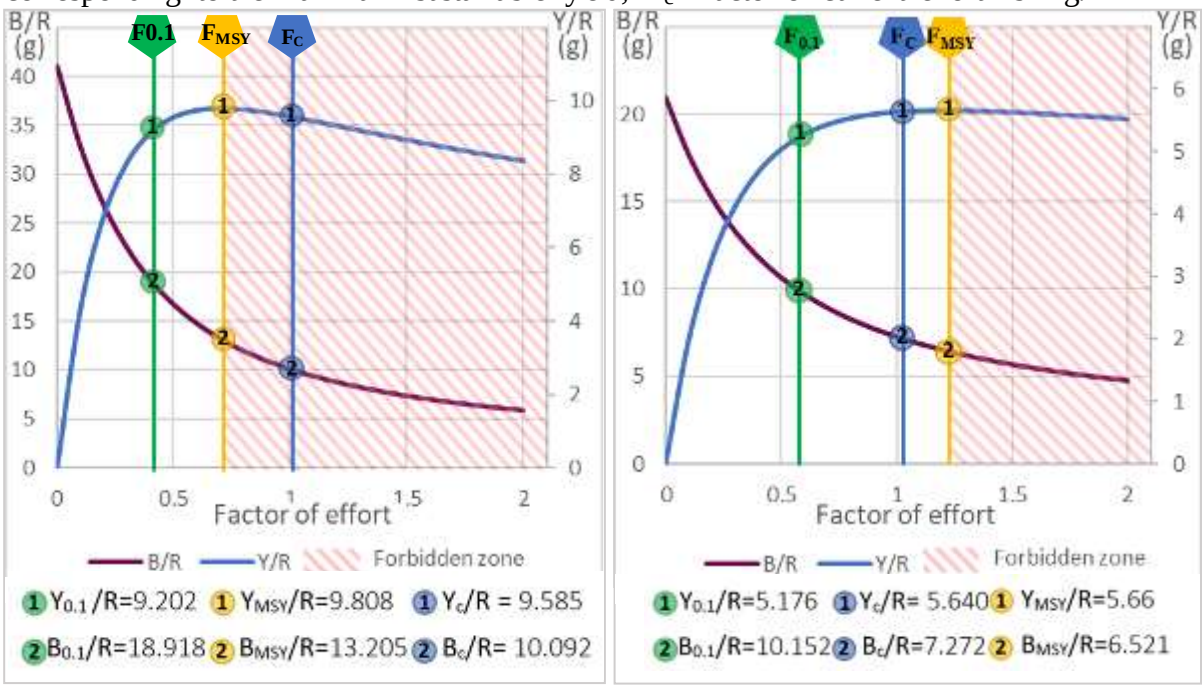


Figure 10. Yield per recruit (Y/R) and biomass per recruit (B/R) according to the effort factor with constant natural mortality (M_{cte}) (left) and with variable natural mortality (M_n) (right).

At the first glance on the graphics in figure 1 we can already see that the results differ according to the natural mortality used, we can also see that with M_{cte} the current factor effort (F_c) exceeds the maximum sustainable yield effort factor (F_{MSY}) and is positioned in the forbidden zone. On the other hand with M_n the current effort factor is positioned before the maximum sustainable yield and thus it is not in the forbidden zone, moreover, according to the values with M_{cte}, $F_{0.1}$ = 0.400, this value imply that to maximise a sustainable exploitation we would have to reduce fishing effort by 60% (to increase Bc to 16337.6 tons), that said, with M_n, $F_{0.1}$ = 0.54, so to have a sustainable exploitation we would need a reduction of 46% of fishing efforts (to increase the biomass to Bc = 14848.9 tons).
To investigate the occurrence of an overexploitation we used the Froese and Proelss (2012) criteria, and obtained the following, for constant natural mortality (M_{cte}) the results showed an overfished and overfishing state (dark grey), on the other hand, with variable natural mortality (M_n) the results showed a not overfished and not overfishing state (light grey).

Conclusions

According to the previous results biomass assessment is largely sensitive to mortality values. By comparing biomass assessment using M_{cte} with biomass obtained using M_n we obtained an estimated shift of the biomass value of 1949.8 tons, (Bc = 8686.6 tons with M_{cte} to Bc = 106364.4 tons with M_n). Additionally, results obtained led to different stock evaluations and even different suggestions. Consequently, while using M_{cte} the results pointed that the stock was in a state of overfishing and overfished, while when using M_n, the results showed a state of not overfished and not overfishing. Also, when using M_{cte} the results suggested a

fishing effort reduction of 60%, while with M_n the results suggested a reduction of fishing effort of 46% (to increase biomass to Bc = 14848.9 tons). Since mortality values changes with age and considering the impact of the natural mortality used on the biomass assessment, we therefore suggest the use of variable natural mortality when conducting similar procedures.

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A NEW METHOD BASED ON INFORMATION PROVIDED BY GATHERERS AND GPS SENSORS FOR A SUSTAINABLE MANAGEMENT OF AROMATIC AND MEDICINAL PLANTS

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Abstract

The state of preservation of MAP in Albania imposes a true reflection on how their operations move towards a sustainable management. The sector was previously managed by the state andafter 1991 it passed to private leadership, while resources were still publicly owned. Even there are the regulations and contracts between public administrators and private firms, the "free" access of gatherers and the pressure of the competition between economic officers led by increasing the supply create a real problem in terms of the sustainability of these resources. The objective of this study is to show that based on the information of the gatherer and the GPS traces of the activity, we can identify the habitats utilized through a cartographic image. Determining the action of gathering comes down to indirectly detecting the location of a plant species. Applying successive filters for instantaneous speed, spatial-temporal density, surface area and average of angle variations is used to model the picking area (pa) which, by aggregation on various scales, helps reconstitute the exploited habitat. This model was tested for the plant species of lime blossom. Finally, applying this approach, we can evaluate the state of plant habitats by having regular and easy-to-update automated series as well as spatiotemporal statistical analyzes. The provision of this management tool gives to professionals an objective knowledge base on aromatic and medicinal plants. They can perform extractions from the database to meet the requirements of traceability and preservation of the resource.

Keywords: *Medicinal and aromatic plants, gatherer approach, sustainable management, human sensor [GPS].*

Introduction

The exploitation and utilization of the medicinal and aromatic plants (MAP) is a considerable weight in the global economy. According to Comtrade data the annual growth rate since 1988 is 11% and their value in 2014 reached $ 3 billion (Ilbert & Hoxha, 2016). The increase of the demand has made sector actors concerned about the resource management situation of these resources. Studies show that many habitats are degraded and many MAPs are endangered primarily due to deforestation, overgrazing, competition with invasive alien species, climate change and destructive harvesting practices and overexploitation (Schippmann et al, 2006; SSC / IUCN / TRAFFIC / WWF 2008; Leaman, 2009, CBD 2010). Studies carried out by specialists and organizations are concerned for continuous observation at the spatio-temporal scale for these plants as well as an interdisciplinary and participatory approach of the concerned actors. This lack of information causes for the managers of these habitats a problem of decision-making regarding the plant management and protection, while for the users and value chain actors causes a problem of assessment and sustainability (Schippmann *et al.*, 2006; MPSG / SSC / IUCN / WWF, 2008).

The qualitative studies and the inventories made by the specialists reflect partially the reality on the field. There are no systematic studies that can provide information's on biophysical condition of the habitats and their evolution. Inventories made are few and they do not cover the entire territory of the MAP, or even the cut based upon the administrative boundaries instead of ecosystems. The identification of the habitats, the number of species, identified as determinants of erosion, are neither comprehensive nor systematic. Nowadays the key issue is that there is no real time and space information about the origins of the collected quantity, geographic distribution and the potential of their habitats (USAID, 2010).

The method applied until now to aromatic and medicinal plants is that of the botanical inventory which proceeds by samples. Knowledge of the habitat of the plant is carried out by specialists (foresters, botanists) who operate via the floristic sampling of samples. An extrapolation is then carried out, as a function of the surface and the yields, in the whole predetermined territory (Kutrolli and Ukaj, 2009). This method has now been enriched with geographic tools such as GPS or GIS in order to better locate and observe the test samples (Dano, et al.2008).

At the technical level, the botanical method used to obtain the situation information and the mapping of exploited habitats requires great efforts regarding the specialists needs, financial resources, and is limited on time. Today, several research teams rely on geospatial tools to map and analyze the spatial and temporal interactions between the physical environment, the place of the living and the pressures of climate change and anthropogenic sources to guide the management strategies, the exploitation and sustainable conservation of the biodiversity (Baron *et al.*, 2009; Lamanda *et al.*, 2009, Pasteur *et al.*, 2009). The methods obtained from remote sensing or for spatial observation, offer a great opportunity for the identification and management of natural resources but their applications in the case of the MAP have their limitations. Many species of MAP are under crop canopy and they are not directly visible by conventional methods of remote sensing. In the Nature they occupy not particularly homogeneous surfaces. Although the detection methods are carried out on the scale of a plant, most of the MAPs are of a sub metric scale; in addition, some plants have a very similar morphology to sheet sometimes. As for the botanical sampling method, even with these methods, we face some problems related to human resource needs as well as technical problems and even financial.

In other areas such as the identification of fauna habitats, biologists use indirect methods. Using the GPS technology the knowledge of predator movements also enables identification of prey habitat (Soisalo & Cavalcanti, 2006). Also, for the optimization of urban transport, researchers analyze the behaviors of different social groups relying on the concepts of "Time Geography" approach, and by analyzing GPS traces of their trajectories (Asakura & Hato, 2009, Klein & Schneider, 2011).

In this context, using the gatherer as a source of information and relying on the concepts of "Time Geography" to analyze its GPS activity traces, for us was another way to map and to know the habitat exploited. Assuming that the gatherers indicate where the plants are, the idea is to follow them in order to share and structure their information. The approach is participatory (crowd sourcing) involving gatherers as information contributors. Their GPS tracks and their knowledge (name, quantity, etc.) contribute to identify and to characterize the gathering area (ZA). The aggregation in time and in space of the information allows designating plants in their ecosystems.

So, our goal was to create an alternative method that spotted the time and space recognition of the exploited habitats through information and analysis of GPS traces taken by the plant gatherer. Determining GPS tracks of the action of gathering comes down to indirectly detecting the location of a plant species. GPS tracks are processed and analyzed by a model

that applies a set of filters to retain only the portions of the track concerned by gathering activities in the strict sense of the term.

Material and Methods

Filters construction

The theoretical reflection led us to develop an experimental model according to the specificities of the harvesting of wild plants. In order to "retain" only the GPS traces of the gathering action in the strict sense, the concepts of the "Time Geography" approach were used as a basis for constructing the four filters: instantaneous velocity, spatio-temporal density, the surface and the mean of the angle variations (FIG. 1).

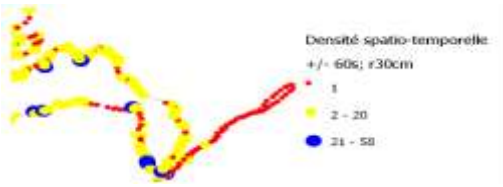
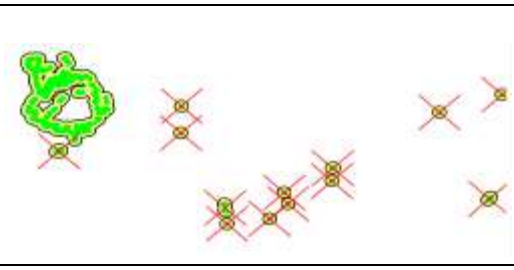
The first filter is the instantaneous speed. It serves to eliminate items that have a signature similar to the action of the gather in the strict sense.	
The second is the filter of the spatio-temporal density. It serves to distinguish items that have a specific signature on the work of the gatherer in the strict sense.	
The third is the filter of the surfaces. It starts from the premise that only the interconnected sites are representative.	
The fourth is the filter of the average variation of the angles. A methodological hypothesis that: more the variations angles are chaotic and more intense are the actions of the gatherer.	

Figure 1.Methodological filters to identify the range of MAP

Applying successive filters for instantaneous speed, spatial-temporal density, surface area and average of angle variations is used to model the picking area (pa) which, by aggregation on various scales, helps reconstitute the exploited habitat.

Translation of filters into spatial SQL language and choice of plants to be experimented

The design of the theoretical model was translated into SQL language and incorporated in a spatial database to facilitate automated data processing.

Three steps were carried out: The first step is to encode this information into a spatial database called Postgresql / PostGIS. The conversion of the acquired data was made thanks to the free library GDAL and notably OGR. The second step involves processing the structured data according to the spatio-temporal filters constructed for our model. Each filter corresponds to a spatial SQL query. Automatic processing was possible thanks to a free programming language. Finally, the last step is the visualization of the processed data. This automated model is coupled to declarative information gatherers: in each collection is given

the name of the plant and the daily quantity picked. This information provides the density of the harvesting areas and their evolution through time.
This was the object of a statistical analysis of all the registration points of the GPS. The results were consistently faced with the reality on the field documented using delocalizing photos, or even some videos. This helped to identify and refine empirical parameters [resulting intervals and thresholds] to apply to filters according to the plant species or type of plant [tree, shrub, herbaceous]. To verify the feasibility of the data method with the following characteristics were used 10 samples (GPS tracks), 4 different gatherers, 3 plants (lime, sage, rosemary) and four distinct geographical zones: 1 zone in the district of Përmet (Albania); 2 zones in the district of Tepelenë (Albania) and one area in the north-est of the department of Herault (France). In this communication, we will give the results of the linden plant in the Tepelena area.

Results and Discussion

Final results of application of the model: the case of linden in Albania

Figure 2 provides information about the harvester (name, village and commune), harvesting data (type of plant, date, time, duration and harvested quantity, background maps contextualizing the action) and represents on a background of the satellite map the recorded gross path, at three different spatial scales.

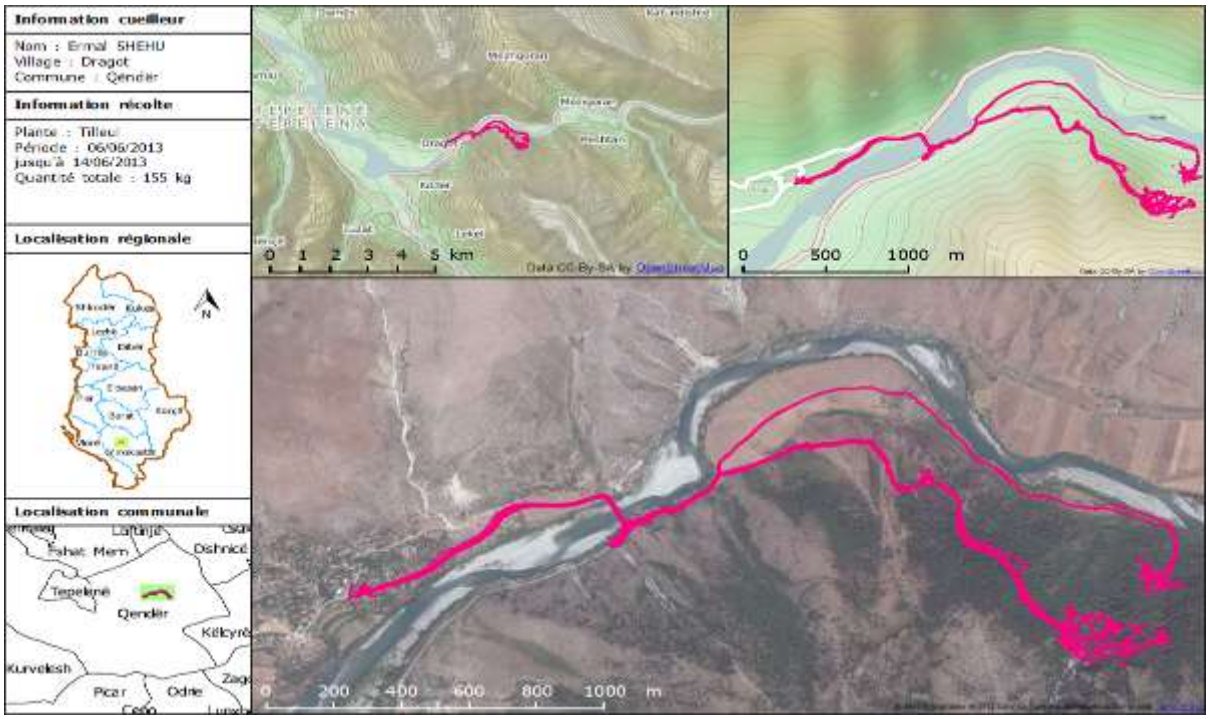


Figure 2. General information and set of one week gatherer raw tracks.

The application of the instantaneous velocity filter divides the points into two characteristics: points which have a velocity of less than 0.3 m/s are stationary points whereas points which have a velocity greater than or equal to 0.3 m/s represent the actions of displacements. Thus, the stationary points characterizing the picker's immobility are within the velocity range of [0 - 0.3 m/s]. For a total of 251037 points, 209768 points are stationary, or 83.07%.
The setting of the thresholds for the filters of spatio-temporal density, the surface area and the average of the angle variations, is a work carried out in a specific way. In the case of linden

the specific interval is ≥ 600 points. In order to reduce the uncertainty in the definition of the picking area, the intersection of the points of the two filters cleans all the points of displacement as well as those which have a density located outside the points of the picking action. But these points may belong to several other types of actions that are not in the collection area.This is where the surface filter makes a significant contribution. The size of the polygons makes it possible to distinguish surfaces with artefacts, but there is always uncertainty. We applied a buffer zone of 20 m for the surface filter and then we calculated the values associated with each polygon.

Accordingly, in order to define the presumed areas of the picking area, we apply an additional constraint: all the surfaces (light blue color) which are ≥130% of the minimum area and which have an average of the angle variations ≥ 50 (Figure 3).

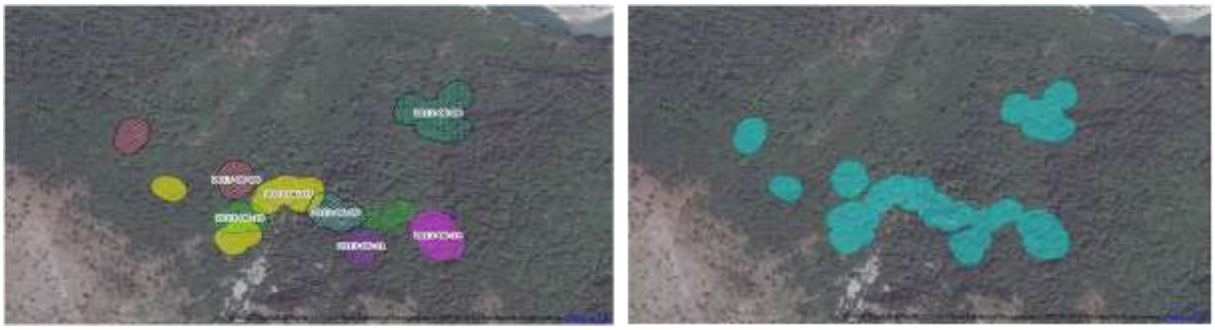


Figure 3. Areas of gathering with their timestamps (left)
Figure 4. Mapping the exploited habitat aggregating time and space in an ongoing process (right)

Finally, by aggregating the selected polygons we can build the habitat exploited (Figure 4).

Conclusions

We consider the main objective relative on the feasibility functional and the filters developed as operational. It is therefore possible to reconstruct a habitat of plants by aggregation of GPS traces filtered. In parallel it should then be considered a method of continuous improvement through an iterative development cycle including regular confrontation of the habitat area obtained by the model and reality. To receive, analyze and enhance data establishing an observatory dedicated to MAP seems to be the next logical step. Only an operational observatory, as a knowledge base and decision support tool, will permit to manage more effectively the MAP. To protect the MAP and their habitats is necessary to have update information and follow it evolution in time. For this reason the habitat mapping through the observatory contribute in information is very important. This work has already set up an early IT infrastructure that can be considered as the foundation of the future information system of the observatory. The process of importing data already operates automated. Management scalability of the observatory will be obviously a critical issue. The treatment of several tens of tracks by days obviously does not require the same infrastructure as the data from several thousand gatherers. It is important to note that the data sources are always saved to be reused after the evolution of the process data [change filters].

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ANIMAL HUSBANDRY

CONSIDERATIONS REGARDING THE MOUNTAIN PRODUCT IN ROMANIA: PRESENT STATE AND PERSPECTIVES IN RELATION TO THE CATTLE BREEDING ACTIVITY

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Abstract

The paper aims to present the situation of the mountain product in Romania, between June 2017 and March 2020. This work is based on statistical data provided by the National Sanitary-Veterinary and Food Safety Authority, the National Institute of Statistics and the National Agency of Mountain Area (ANZM). These data were processed within the National Agency of the Mountain Area (ANZM), in the following indicators: number of counties with mountain area, number of administrative-territorial units, total area of the mountain area (km^2), number of animals in the mountain area (cattle, sheep, goats, pigs), number of mountain products and categories of mountain products certified in Romania. Compared to the year 2017, the number of mountain products that benefited from the qualitative mention of mountain product increased, reaching 2020, to 491, according to the National Register of Mountain Products. This shows the recognition of the quality of the products obtained in the mountain area of Romania. The bovine species, with the two main productions, milk and meat has a significant influence on the mountain product at national level, but also in the Dornelor Basin. Analyzing the graphical representation of the mountain product we find that dairy products are the most widespread (50%), followed by vegetable products by 30%, and followed by bee products by 17%.

Keywords*: mountain product, Romania, livestock, bovine.*

Introduction

The mountain is a very important source of air, water and food all over the earth. Over 80% of the fresh water comes from the mountain area. In the world, mountains occupy 24% of the surface of the earth, respectively 40 million km². In the mountains, 12% of the world population lives, and 14% live in the immediate vicinity of the mountains. Europe is covered by 40% of the mountains, inhabited by 20% of the total population of this old continent (Rey, 1985). Mountain areas are, in general, rural and are characterized by natural limitations of agricultural productivity, which lead to reduced agricultural production, caused by adverse climatic and biophysical conditions for optimal agricultural activities (Law no.197 / 2018 - Law of the mountain). The mountain area of Romania represents a disadvantaged national territory, with high economic, social, and cultural potential, which needs a different approach regarding the policies and strategy of development and protection of these mountain areas (Maruşca, 2018). The recognition of the quality of the products obtained in the mountain area can be made by assigning these products the optional mention of mountain product, which is regulated at European and national level by normative acts, respectively procedures for awarding at national level the mention of mountain product quality (Apetrei, Surdu, 2017).

Material and method

The following indicators were used to characterize the situation of the mountain area in Romania: number of counties with mountain area, number of Administrative-Territorial Units in the mountain area, total area of the mountain area (km^2), number of animals in the mountain area (cattle, sheep, goats, pigs) (Statistical Yearbook of Romania 2016-2019, http://www.insse.ro), number of mountain products from Romania and the categories of products that benefit from this mention of quality (ANZM-National Register of Mountain Products). The period analyzed was June 2017- March 2020, and the data were provided by the National Sanitary-Veterinary and Food Safety Authority and also by the National Institute of Statistics; these data were processed within the National Agency of the Mountain Area.

Results and discussion

The mountain area of Romania (Fig.1) has a total area of 71,381.48 km^2, and comprises 658 Territorial Administrative Units, respectively 27 counties with mountain area, which represents 30% of the territory of the country, of which 577 communes, 81 cities and municipalities and 3536 villages. The number of animals from the mountain area of Romania is presented in table 1.



Figure1. Representation of the mountain area in Romania
Source: Ministry of Agriculture and Rural Development

Table 1. Evolution of livestock during the period 2017-2019 in the mountain area of Romania

Current number	Indicator name	2017	2018	2019
1.	Cattle (number)	653,069	634,882	614,303
2.	Sheep (number)	2,753,399	2,753,492	2,587,345
3.	Goats (number)	255,572	243,523	211,992
4.	Pigs (number)	260,054	198,848	254,509

*Source: National Agency of the Mountain Area

Analyzing the information in Table 1 we find that all species have a decrease in the number of herds in 2019 compared to 2017. The explanation lies in the fact that the pasture in the mountain area is poor, which causes the breeders to reduce the number of herds. In order to be able to obtain reasonable productions, the pasture must be artificially improved. The sheep behave better on the pasture than the bovine species for which the grass must be at least 10-15 cm high in order to graze (Maciuc, 2006). The pig species is omnivorous and often procuring cereals to provide the necessary food is a problem. The latest European Regulation on quality schemes for agricultural products and foodstuffs, in addition to underlining the importance of consolidated optional certification schemes (PDO, PGI, TSG), confirms the importance of specific origin for rural areas. The European Parliament drew attention to the need to improve mountain and island areas and introduced the "optional quality terms". Delegated Regulation, EU no. 665/2014, defined the conditions for the use of the term "Mountain product". According to the current guidance, this optional term provides a labeling scheme that allows the promotion of qualified mountain production. According to the aforementioned Regulation, the following product categories can benefit from the qualitative mention of mountain product: animal products, animal feed, bee products, vegetable products, processed products.

In the case of products of animal origin, the maintenance of quality mountain product can be attributed to the products obtained from animals from mountain areas and which are processed in these areas, as well as for animals raised at least the last two thirds of life in the mountain area and for transhumant animals, raised at least a quarter of life in transhumance and who grazed in the mountain pastures. In the case of animal feed, this mention is attributed to the food that comes from the mountain area when the proportion of the annual feed ration that cannot be produced in the mountain area, expressed as a percentage of dry matter, does not exceed 50%, and in the case of ruminants, 40% .The optional mention of mountain product can be attributed to bee products if the bees have only collected the nectar and pollen from the mountain areas.

In the case of vegetable products they must be grown in the mountain area in order to be labeled as a mountain product. The processed products can benefit from this mention of quality if the processing takes place in the mountain area and not more than 30 km from this area. In Romania, the Government Decision no. 506 of July 20, 2016 on establishing the institutional framework and some measures for the implementation of the delegated Regulation (EU) no. 665/2014 of the Commission of March 11, 2014 supplementing Regulation (EU) no. 1.151/2012 of the European Parliament and of the Council regarding the conditions for the use of the optional quality mention "mountain product", by which the National Agency of the Mountain Area is designated as the competent authority responsible for elaborating the procedure for verifying the conformity of the data contained in the specification tasks in order to grant the right to use the optional mention of "mountain product" quality and to verify compliance with European and national legislation by the economic operators who have obtained the right to use that mention The certification of the mountain product in Romania is regulated by Order no. 585 of December 20, 2019 for

amending the annex to the Order of the Minister of Agriculture and Rural Development no. 52/2017 regarding the approval of the Procedure for verifying the compliance of the data contained in the specifications for granting the right to use the optional mention of "mountain product" quality and for verifying the compliance with the European and national legislation by the economic operators who obtained the right to use of the respective statement, which regulates the competent authority that verifies the specification, the national register of the "mountain products" will be updated and completed by the National Agency of the Mountain Area, the following terms are defined: the mountain area, "mountain product", the applicant, the specification. The high quality of "mountain products" (dairy products, meat products, etc.) is given by the floral polymorphism of the meadows, the absence of the use of chemical substances, the unpolluted environment. Demographic growth and the existence of a significant market segment interested in "healthy" mountain products ensure sustainability. The key is to provide motivating prices, especially for milk and meat as raw materials. The type of "savage" capitalism installed by "processors" and other intermediaries, has ridiculously low prices that have led to deterrence, poverty, abandonment of agriculture, migration of young people from rural areas and serious damage to the functionality of the biodiversity of the grasslands in the mountain areas, this system being compromised and "toxic" to the mountain economy (Șut-Gherman, 2006). There is an urgent need for a legislative alternative, which will ensure a separation of the "mountain product", the significant volume and continuity throughout the year, the quality and health guarantees for consumers. An innovative associative-cooperative system adapted to realities, organized at the level of the mountain basins for "niche mountain products" and niche consumers, with local brands could offer the recovery of the development and determine a sustainable growth of the mountain area (Rey, 2014). In Romania, between June 2017 and March 2020, 129 decisions were issued by the National Agency of the Mountain Area, using the optional mention of "mountain product' to economic operators in 21 counties with mountain area, respectively 491 mountain products (Table 2) guidance, this optional term provides a labeling scheme as:

- Milk and dairy products;
- Meat and meat products;
- Vegetable products;
- Bee products;
- Fish products;
- Bread, bakery and pastry product.

Table 2. The total number of mountain products that have obtained the right to use the optional mention of "mountain product" in Romania

Categories of mountain products	Number of products	Counties	Total no mountain products	Number of decisions granted
Milk and dairy products	246	21	491	126
Meat and meat products	9			
Vegetable products	144			
Bee products	85			
Fish products	6			
Bread, bakery and pastry products	1			

Source: National Register of Mountain Products-ANZM

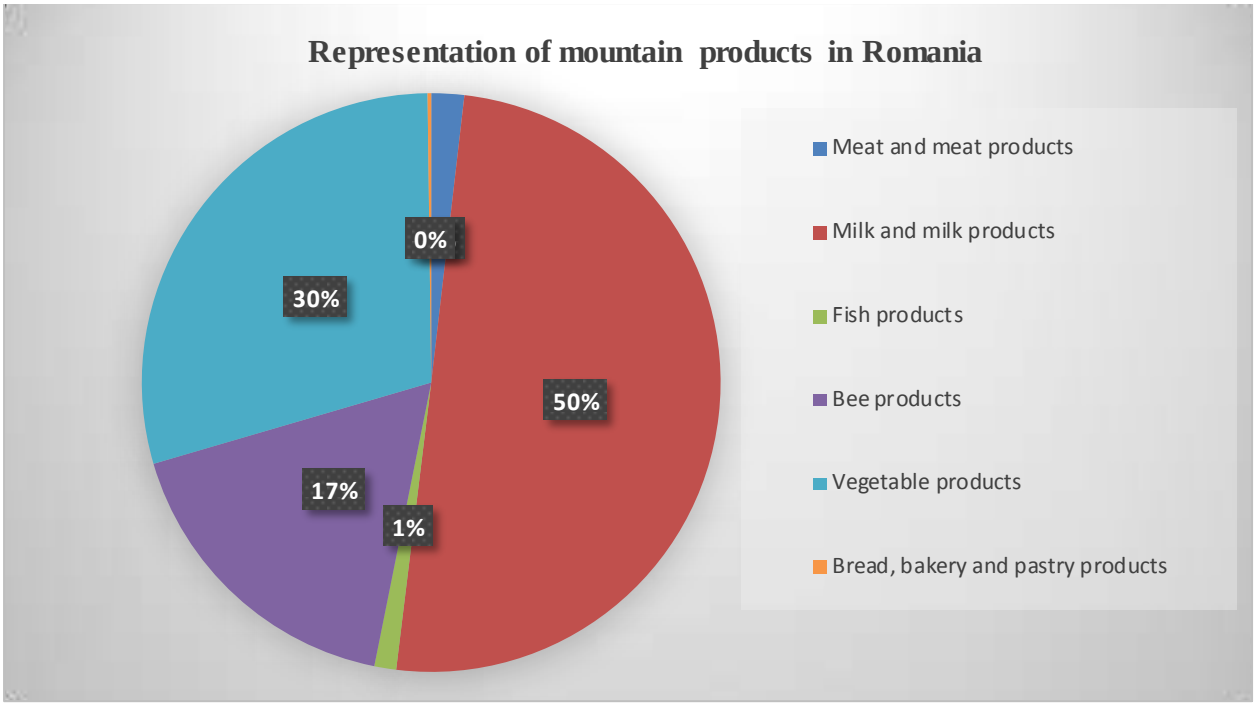


Figure 2. Representation of the mountain product at national level

Regarding the bovine species, the two main milk and meat products have a significant influence on the mountain product at national level, but also in the Dornelor Basin. We can provide some examples regarding mountain products made from milk: Ţvaiţer „Călimani", Cheese "Călimani", Smoked cheese "Călimani, but also for mountain products made from meat: Sausage grill Angus, Sausages Angus spicy, Angus pastrami, Parisian Angus, Burger Angus. Analyzing the graphical representation of the mountain product we find that milk products are the most widespread (50%), followed by vegetable products by 30%, followed by bee products by 17%.

Conclusions

Mountain areas should benefit from a specific policy defined in accordance with the principles of sustainable development, which ensures the needs of the present without compromising the chances of future generations. The strategic orientations aim to reduce the imbalance between the most favored regions and the disadvantaged mountain regions, marked by permanent natural constraints, aiming at the whole economic, social, cultural and environmental problem. The local products offer economic, social and environmental benefits, they mainly support the local economy (by providing jobs and incomes that are most often spent locally), develop a direct trust relationship between consumers and producers, promote social cohesion and community spirit, encourage the community to adopt ecological behavior, promote a sustainable and circular production system, reduce outsourcing in transport and contribute to maintaining biodiversity and developing endangered varieties. These are the most relevant aspects for mountain products, which have specific characteristics given by the production environment, the quality of natural resources, the traditional techniques and the know-how used for their production and processing. The mountain products are an integral part of the image of the mountain territories and are therefore an advantage for their development. The bovine species, with the two main productions, milk and meat has a significant influence on the mountain product at national level, but also in the Dornelor Basin. Analyzing the graphical representation of the mountain product we find that

dairy products are the most widespread (50%), followed by vegetable products by 30%, followed by bee products by 17%.

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EFFECT OF *BACILLUS* SPP. FOOD ADDITIVES ON WEIGHT IN WEANED PIGLETS

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Abstract

To improve pig farming and reduce losses in the most vulnerable categories of pigs, either in suckling piglets or weaned piglets, in addition to basic nutrients in complete mixtures, piglets are given a large number of feed additives for different purposes. Feed additives are substances that are added in very small quantities in order to improve the health of animals. In recent decades, scientists and experts have been paying more and more attention to the so-called "alternative" additives, of which enzymes, organic acids, probiotics and prebiotics are the most frequently mentioned. Due to many unknowns that accompany these products in their use, especially in our country, we have selected the probiotic based on bacteria *Bacillus* spp. to test it and its effect on the weight of piglets. The research included piglets of the Swedish Landrace breed, and was conducted in three separate experiments (I, II and III) in the same facility on a mini-farm. In the first and second experiment, 56 piglets were used (28x2), in the third there were 88 piglets (44x2). In each of the three experiments two groups of weaned piglets were formed. The first one (I) was without probiotic (control group) and the second one with probiotic in their food. In feed, the probiotic was in the concentration of 0.01%. Measurements were made every 14 days until the body weight of the animals reached 25 kg. The weight of the weaned piglets ranged from 8-25 kg. The average body weight of piglets that took the additive was higher by 1.08 kg.

Keywords: *Bacillus spp, additive, piglets, weight*

Introduction

Suckling piglets are the most vulnerable category in pig farming. Weaned piglets are the second category when it comes to vulnerability, since it is necessary to transition gradually from breast milk to concentrated feed with as less stress as possible. Changes in accommodation (environment) and diet cause stress in piglets, which usually manifests in diarrhea. Diarrhea is caused by disorders of microbial balance in the intestines. Nowadays early weaning is common practice to ensure productive yield of pig farms (Barba-Vidal *et al.*, 2018)

The use of antibiotics showed some negative effects. Because of that, some nutritionists advise that natural additives should be used (like probiotics). The main goal of probiotics is to prevent negative effect on balance of the gastrointestinal tract (Hong *et al.*, 2005). Study shows that a mixture of bacteria and yeast has the potential to be used as a probiotic dietary supplement in grower pigs (Giang *et al.*, 2011). Probiotics are the microorganisms that ensure health benefits to the receiver. Among others, *Bacillus* bacteria have strong scientific data which substantiates the validity of the use as preferred probiotics (Suva *et al.*, 2016). Members of bacteria genus *Bacillus* have been used as probiotic supplements in human and animal food for a long time (Cutting, 2014).

For example, Paciflor C-10 has *Bacillus cereus* as its active constituent (European commission, 2003). Combinations of probiotics, oligosaccharides, and berberine had the same effect as antibiotics on reducing diarrhea rates and improving gut microflora for pigs (Fan *et al.*, 2015). *Bacillus* strains are more stable during processing and storage of food and pharmaceutical preparations, making them more suitable candidate for health promoting formulations (Elshaghabee *et al.*, 2017). Natural herbal products are often used as antibacterial agents, antimycotics, antiparasitics, disinfectants and immunologic adjuvants (Grčak *et al.*, 2018).

Probiotics indirectly influenced on the weight gain and suppression of certain pathogenic microorganisms, all of which resulted in an increase in weight of pigs and production results (Conway, 1994).

Probiotics based on *Bacillus* had beneficial effects on weaned piglets challenged with *Salmonella enterica* and *Escherichia coli*, and can be used as alternative to antibiotics (Ahmed *et al.*, 2014). Studies show that oral administration of *Bacillus licheniformis-B. subtilis* mixture (BLS mix) reprograms the gut microbiota and helps goblet cell function to ameliorate enteritis (Zhang *et al.*, 2017). Experiments suggested that probiotics could have beneficial effects on growth performances, blood parameters and immunoglobulin G stimulation of weaned pigs (Dlamini *et al.*, 2017). Probiotics showed therapeutic potential for diseases, including some immune response-related diseases, such as allergy, eczema and viral infection (Yan and Polk, 2011).

The probiotics based on Pediococcus acidilactici and *Bacillus subtilis* showed positive results on piglets in the nursery phase (Silva *et al.*, 2006). *Bacillus sp.* have been studied for many years and developed as commercial products for animal use. Recent studies have indicated that probiotics positively affect the overall health status of their animal hosts (Mingmongkolchai and Panbangred, 2018). Paciflor was also able to reduce ammonia concentration in blood (Scheuermann, 1993).

Preweaning supplementation of *Bacillus subtilis* C-3102, positively affected some gut health parameters while postweaning supplementation improved the feed: gain ratio (Michiels *et al.*, 2016). Probiotic *Bacillus* positively affects the intestinal epithelial cell barrier and immunity system of piglets (Du *et al.*, 2018). Due to robustness of *Bacillus* bacteria in withstanding high temperatures, these probiotics are becoming increasingly popular for use in animal feed (FAO Animal Production and Health, 2016). In 2002, probiotic Paciflor C-10 was removed from distribution because it was discovered that it can produce enterotoxins who could lead to food poisoning (Hong *et al.*, 2005).

The experiments conducted during this study were set in order to examine the effects of the use of probiotics based on *Bacillus* spp., known under the trade name Paciflor, a nutritional additive in complete mixtures for weaned piglets that weigh from 8-25 kg.

Material and Methods

The concentration of *Bacillus* in used probiotic is ten billion (10^{10}) live bacteria in a gram of the substance. The research which included piglets of the Swedish Landrace breed, was conducted in three separate experiments in the same facility on a mini-farm. The first two experiments lasted 52 feeding days, and the third one lasted 47 feeding days, during this time, the piglets reached an average of 22 kg ± body weight. In experiments I and II, each group consisted of 28 piglets, and 44 in third experiment (III), wherein the gender ratio in the groups was the same. The animals were fed with powder mixtures from feeders, and had water supply in from of the so-called nipple drinkers. In each group there was one nipple drinker, 15cm above the ground level.

Results and Discussion

In pig farming, rearing piglets up to 25 kg of weight can cause many problems that are manifested in retarded growth, diarrhea, usually the first two weeks after weaning. Table 1, Table 2 and Table 3 shows the body weight of piglets in the groups in experiment I, experiment II and experiment III.

Table l. Weight of piglets in Experiment I, kg

Group	Variations measures				Index %
	n	X, kg	S	C.V.	
on the 1st day of experiment					
I	28	8.90	1.12	12.58	100.00
II	28	8.71	0.74	8.46	97.86
on the 14th day of experiment					
I	28	9.68	2.05	21.22	100.00
II	28	9.89	1.14	11.59	102.17
on the 28th day of experiment					
I	28	15.66	2.09	13.26	100.00
II	28	16.50	2.12	12.83	105.36
on the 42nd day of experiment					
I	28	21.11	2.74	12.96	100.00
II	28	21.98	3.46	15.75	104.12
on the 52nd day of experiment					
I	28	25.35	3.22	12.70	100.00
II	28	26.95	4.62	17.13	106.31

I-control group, without probiotics; II-experimental group, with probiotic supplemented; n- number of animals in the group; X- the mean weight, kg; S- standard deviation; C.V.- the coefficient of variation; Index expressed in %.

The data on the weight of piglets during the experiment indicate, on the basis of similar coefficients of variation, that there has not been extreme delays in rearing piglets.
The data obtained by the analysis of variance indicate that there has not been statistically significant differences in the groups compared, in terms of body weight during the periods examined.
It has been found that probiotics produce effects by lowering the pH of the intestinal content, occupying the surface openings of the cell membrane.

Probiotic is considered to be successfully implemented if it has the ability to pass through the stomach and then survive and reproduce in the small intestine, and live in the intestines for several days.

Table 2. Weights of piglets in the II Experiment, kg

Group	Measures Variations				Index %
	n	X	S	C.V.	
on the 1st day of experiment					
I	28	8.84	0.87	9.79	100.00
II	28	8.89	0.94	10.53	100.57
on the 14th day of experiment					
I	28	11.09	1.36	12.27	100.00
II	28	10.92	1.57	14.35	98.47
on the 28th day of experiment					
I	28	16.09	2.38	14.82	100.00
II	28	15.91	2.44	15.31	98.88
on the 42nd day of experiment					
I	27*	20.82	3.20	15.38	100.00
II	27*	21.61	3.40	15.72	103.79
on the 52nd day of experiment					
I	27	25.80	4.28	16.61	100.00
II	27	26.51	4.25	16.02	102.75

I - control group – without probiotics; II - experimental group – with probiotic supplemented; n - number of animals in the group; X - the mean, kg; S - standard deviation, C.V. - the coefficient of variation; Index expressed in %.*In Experiment II over the period from 42nd to 52nd day, one piglet was eliminated from each group

Table 3. Weights of piglets in the III Experiment, kg

Group	Variations measures				Index %
	n	X , kg	S	C.V.	
on the 1st day of experiment					
I	42*	8.77	1.47	16.74	100.00
II	44	8.92	1.42	15.88	101.71
on the 14th day of experiment					
I	42	10.89	1.82	16.57	100.00
II	44	11.15	1.57	14.14	101.55
on the 28th day of experiment					
I	42	16.24	2.75	16.92	100.00
II	44	16.82	2.13	12.71	103.57
on the 42nd day of experiment					
I	42	23.62	3.66	15.51	100.00
II	44	24.32	3.24	13.31	102.96
on the 47nd day of experiment					
I	42	26.92	4.18	15.52	100.00
II	44	27.86	4.13	14.84	103,49

I-without probiotics, II – with probiotic supplemented, n - number of animals in the group; X - the mean, kg, ; S - standard deviation; C.V. - the coefficient of variation; Index expressed in %. * In Experiment III two piglets were eliminated (in control group-I) from beginning

In the III experiment, in terms of the weight of piglets at the beginning of the experiment, there were slight differences as a result of eliminating some heads at the beginning of the research, due to death, but the animals were equable nevertheless, because the coefficients of variation in I and II groups of piglets were almost equal and amounted to 16.74%, and 15.88% (Table 3).

In experiment III, piglets were measured to have around 25kg in only 47 feeding days. There were no statistically significant differences between experiment I and experiment II (with and without probiotic).

Conclusion

The weight of piglets obtained in the experiments indicate that the addition of probiotics to feed increases the weight of piglets, but that the differences are not statistically significant. However, since the probiotic added has shown a positive effect on the utilization of nutrients from the feed and conversion, and has not shown a negative effect on the weight. The weight of piglets between the sample (II) and control (I) groups in all three experiments did not show a statistically significant difference. But in the first experiment, piglets that took probiotics had a higher body weight of 1.6 kg, in the second experiment 0.71 kg, and in the third experiment 0.94 kg. The average for all three experiments was 1.08 kg of weight in the piglets that had probiotic with *Bacillus* spp. in their food. Respectively, these piglets had a higher growth of 5.5%. The usage of this probiotics must be based on scientific evaluation. The main goal is for the role of food additives in intestinal tract to be based on the principles of microbial ecosystems in the interconnectedness of the host and bacteria.

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STUDY ON THE EVOLUTION OF PRODUCTION AND PRODUCTIVE PERFORMANCE IN MEAT CATTLE: ABERDEEN ANGUS AND CHAROLAISE IN THE PERIOD 2017-2019 IN THE AREA OF NEAMT COUNTY (ROMANIA)

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Abstract

In this paper we set out to present the results of a study on the evolution of cattle herds and productive performance in the Aberdeen Angus and Charolaise breeds included in the Official Performance Control for meat production, period 2017-2019. For this, information was used from the Associations, accredited for the Official Control of the Performance of Meat Production and for the management of the Genealogical Register for the Aberdeen Angus and Charolaise breeds. The obtained results highlight an ascending evolution of the cattle herds included in the C.O.P. from the two breeds respectively Aberdeen Angus, from 746 heads in 2017 to 1146 heads in 2019, which represents an increase of 53.61%, and for the Charolaise breed, the increase was 53.84%. The herds included in the COP in the Aberdeen Angus breed are significantly higher (93.1%) compared to those in the Charolaise breed (6.9%). Regarding the average daily increase in g / day (smz) compared to the two Aberdeen Angus and Charolaise breeds, the following was found: at the age of 7 months (G200) the Aberdeen Angus breed had the value of 717 g / day, and the Charolaise breed the value of 826 g / day, at 10 months (G300) the Aberdeen Angus breed 982 g / day, and the Charolaise breed 996 g / day and at the age of 12 months (G365), the Aberdeen Angus breed 929 g / day, and the Charolaise breed 1269 g / day. From here, we can conclude that the two breeds obtained results according to the objectives of the breeding program.

Key words: *Neamt, Aberdeen Angus, Charolaise, C.O.P., smz (average daily increase).*

Introduction

At present it can be seen significant growth of global and national beef requirements, issues dictated by:

- Orld population growth;
- The accentuated tendency to increase the standard of living and the purchasing power of the consumer (Coman, I.T.; Vidu, L; Marin, M.P.; Ștefan, G.; Mărginean, G.E., 2019);
- low weight of beef in relation to meat consumption on the head of inhabitant (ASR-2019), pg 764;
- he increasing orientation of consumers towards beef, superior in terms of quality compared to other meat sources (Bulgaru, M., 1996);

  limited possibilities for qualitative production of meat from meat and meat-milk breeds (Creangă Șt., Maciuc V. 2009);

- Given the growing demand for meat, meeting this goal can be achieved by increasing the number of cattle and their genetic improvement through science and advanced technologies (Ivancia, Mihaela - 2007).
- Globally, meat breeds aimed at meat production are constantly evolving, with the Aberdeen Angus breed accounting for 70% of their total, being the most widespread and appreciated meat breed in the world.
- Romanian farmers are increasingly interested in raising beef cattle because they are easy to raise and get a good price on the European market. The great advantage of beef cattle is related to the very good breeding efficiency and the fact that their care requires lower costs than in the case of dairy cows (Maciuc V., 2012).
- Furthermor, due to the strict conditions of milk production, many farmers give up milk breeds in favor of meat: Charolaise, Limousine, Aubrac or Angus are just a few new breeds of beef cattle that are raised on farms in Romania
- Many beef breeders prefer the Angus breed, a breed formed in Scotland and which gives very good results in Romania. It is an ideal breed for pastures in Romania. It is a very robust breed, very adaptable to extreme temperatures, between -40 ° C and + 40 ° C (Lupan, V., Chilimar, S., Ujică, V., 1997).
- Another aspect that characterizes it is precocity, ie a reduced age at the first mating, at 14-15 months can be mounted. Slightly speaking, calves at birth have a lower weight compared to other breeds of beef cows.
- It is a prolific breed, a calf can be obtained every year, the gestation period is nine months, and the average daily increase is high (1300 grams per day), given that this breed is exploited in an extensive system, ie on pasture. In addition, our pastures cover a large area and are of very good quality (Halga, P. et al., 2005). The Aberdeen Angus breed capitalizes extraordinarily well on the natural and pedoclimatic resources that our country offers (Otiman, Ion, Păun, 1995).
- Regarding the breeding and exploitation of the Charolaise beef cattle breed at European level, a quarter of the cows destined for meat production are of the Charolaise breed. Of course, this is determined by the characteristics of the breed, one of its characteristics is that in Charolais animals the best use of fodder is found, especially the coarse ones (Drinceanu, D., 1994)
- In fact, their ingestion capacity, superior to that of other breeds, as well as their efficient metabolism allow a good capitalization of coarse fodder (straw, grass on pasture, hay), which are also the most economical (Sîrbu, Marcela, 2005). Adult cows weigh between 700 and 1,100 kg, bulls between 1,000 and 1,600, calves for seven months he weighs between 275 and 320 kg, and the bulls between 310 and 360 kg. Charolais meat has specific organoleptic qualities.

Materials and methods

In order to analyze the cattle herds and the productive performances achieved at the Aberdeen Angus and Charolaise breeds, included in the Official Performance Control for meat production, in the period 2017-2019 in Neamț County, a series of indicators were studied such as: the herd of cattle contained in the C.O.P. (Official Performance Control for meat production), age 7 months (G200), smz, age 10 months (G300), smz, age 12 months (G365),

(Acatincăi, Stelian, 2004) but also in terms of combining and correlating the data with the numerous observations from the farms studied.

Table 1. Livestock breeding and exploitation of meat breeds: Aberdeen Angus and Charolaise studied

NO.	Holding	ANSVSA Code	Race	NO. Animals included in the C.O.P.
1	S.C. VALDO FOREST INDUSTRIES S.R.L.	RO1365629003	Aberdeen Angus	119
2	BORDEIANU CRISTIAN	RO1226771004	Aberdeen Angus	177
3	ARTENI MUGUREL	RO1215090149	Aberdeen Angus	45
4	MINUȚ MARIA SIMONA	RO1214750408	Charolaise	35
5	I.I. DĂRLEA OFELIA	RO1243860158	Chaloraise	23

Source / OJZ Neamț / Aberdeen Angus-Romania Association / ACBC Romania Suceava (2017-2019)

The primary data were extracted from the records of the holdings, but also from the records of the administrative offices. They have been systematized, statistically processed and interpreted by methods specific to such research. The statistics, respectively the parameters, which characterize a normal distribution, are on the one hand the average or median, and on the other hand the dispersion indices represented by the variance and the standard deviation of the pursued character. The statistics are denoted in Latin letters: arithmetic mean (), variance (s2), standard deviation (s), and parameters in Greek letters: theoretical mean (μ), variance (ó2) and standard deviation (ó). For this purpose, the computer program S.A.V.C. (Statistics Analysis of Variance and Covariance 2003) to determine the arithmetic mean (), the error of the arithmetic mean (± s) the standard deviation (s), the coefficient of variability (V%) and the tests of significance ANOVA respectively p.

It should be noted that the data analysis was performed in terms of merging and correlating with the numerous observations made directly on farms and with the reporting of the results obtained to the requirements and rules of the European Union (EU).

Results and discussion

Neamț County has large areas of natural pastures, a total area of meadows located outside the 83 Territorial Administrative Units of 98,255 ha. (Source: Directorate for Agriculture and Rural Development). The climate, the relief, the variation of precipitations, the soil, the quality of the forages are some of the great advantages of this area, in order to increase this quality breed in an extensive system. The breeding system of the Angus breed is the extensive breeding in the suckler cow system on pasture, namely, after calving the calf remains with the mother cow until the age of 8-10 months, when it will be weaned (Confederate Margaret, Bazgan Olimpia, MaciucV., 2005). During this time, the calves are on pasture and feed only on cow's milk and grass on the pasture, without additional intake of concentrated feed (Coulon, J. B. et al. 1989). Most of the breeding cattle population stays outside throughout the year, being fed on hay and grass silage in winter. During the studied period we find an ascending evolution of the cattle herds included in the C.O.P. of the two breeds studied: Aberdeen Angus, from 746 heads in 2017 to 1146 heads in 2019, which represents an

increase of 53.61%, in the Charolaise breed, the increase was 53.84%, the herd included in performance testing for meat production was 52 heads in 2017 reaching 80 heads in 2019 (table 2). From the analysis of the data regarding the herds of cattle from the two breeds raised in the zootechnical exploitations from Neamț county and included in the C.O.P. there is an upward trend for both breeds in the period 2017-2019, but the Aberdeen Angus herds are significantly higher (93.474%) compared to those of the Charolaise breed (6.525%).

Table 2. The dynamics of the cattle herds from the Aberdeen Angus and Charolaise breeds included in the C.O.P., at the level of Neamț county between the years 2017-2019

NO	Specification	Years of observation studied		
	Indicators studied	2017	2019	±%2017/2019
1	The total number included in the C.O.P. Aberdeen Angu	746	1146	+53,61
2	The total number included in the C.O.P. Charolaise	52	80	+53,84

Source / OJZ Neamț / Aberdeen Angus Association-Romania / ACBC Romania Suceava (2017-2019)

Regarding the performance for the respective meat production, the average daily increase in g / day (smz) in the Aberdeen Angus breed the results obtained at the age of: 7 months (G200), smz: 717 g / day, at the age of: 10 months (G300), smz: 982 g / day, and at the age of 12 months (G365), smz: 929 g / day. Regarding the performance for the respective meat production, the average daily increase in g / day (smz) in the Charolaise breed, the results obtained at the age of: 7 months (G200), smz: 826 g / day, at the age of 10 months (G300), smz: 996 g / day and at the age of 12 months (G365), smz: 1269 g / day (Table 3).

Table 3. SMZ dynamics in beef cattle included in the COP, by breeds

NO	Specification	Age (days)	smz*
1	Rasa Aberdeen Angus	200	717
2	Rasa Aberdeen Angus	300	982
3	Rasa Aberdeen Angus	365	929
4	Rasa Chaloraise	200	826
5	Rasa Chaloraise	300	996
6	Rasa Chaloraise	365	1269

Source / OJZ Neamț / Aberdeen Angus Association-Romania / ACBC Romania Suceava
*smz *(average daily increase)*, (2017-2019).

Regarding the performance for meat production (smz -g / day) from the data presented in (table 3) a higher growth rate is revealed in Charolais cattle compared to those in Aberdeen Angus breed. Thus, in the Charolaise breed at the age of 200 days, the average daily increase recorded was higher by 29.56%, at the age of 300 days the average daily increase achieved was 1.42% more and at the age of 365 days the average increase daily was higher by 36.59% compared to the results obtained Aberdeen Angus breed, analyzed in this study.

Conclusion

Following the study, the following conclusions can be drawn:
1. In the period studied, 2017-2019, the number of Aberdeen Angus cattle registered in the Official Performance Control for meat production increased from 746 heads in 2017 to 1146 heads in 2019, this increase is due to fluctuation and in general As the price of a liter of milk fell, many farmers began to opt for crossbreeding and buying Aberdeen Angus cattle.
2. An upward trend in the number of cattle in the C.O.P. was also registered in the Charolaise breed, the herd being significantly smaller but with a tendency to increase in the following years and due to the performance for the respective meat production, smz achieved, which is higher than the one registered in the Aberdeen Angus breed (General Association of Cattle Breeders from Romania, 2004).
3. Adult Charolaise cows can reach weights of 1000 kg, intensively fattened youth perform s.m.z. of 1200-1400 g / day, and the yield at slaughter is 60-64%.
4. When crossing with other rustic or dairy breeds, or even with other meat breeds, Charolais bulls bring a dramatic improvement in growth rate and carcass conformation.
As a result, it is the main breed in Europe, used for cross-breeding in the paternal line, to increase meat production.
5. Given that we currently import a significant amount of meat, although we have significant natural and human resources for the breeding and exploitation of cattle in this area of the country, in the future it is necessary: to increase the cutting weight which will lead to high yields. meat in the carcass; integrating cattle breeding into EU rules and standards;
promoting the activity of improving cattle herds in the direction of increasing and improving meat production; financial support for the organization of holdings in associations, in order to represent the interests in relation to the suppliers of inputs and beneficiaries of the realized products; ensuring own revenues by capitalizing on the production of goods for export; ensuring the necessary conditions for the externalization of the animal's production potential; stimulating the growth of herds in mountainous areas that present opportunities in cattle breeding.

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ANTIMICROBIAL RESISTANCE OF STAPHYLOCOCCUS PSEUDINTERMEDIUS

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Abstract

Eleven isolates of *Staphylococcus pseudintermedius* originating from animals were used in this study. Testing was carried out during 2019, and the samples originate from the territory of the city of Banja Luka. For isolation of staphylococci, the standard method of isolation were used, cultivation on blood (5% defibrinated sterile sheep blood) and nutrient agar, with aerobic incubation at 37°C for 24 hours. The sensitivity testing of isolated staphylococci on selected antibacterial drugs was performed by disc diffusion method on Mueller-Hinton agar, by direct suspension of colonies at a concentration 0.5 McFarland, and results were interpreted according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) from 2008 for oxacillin and vancomycin and from 2014 for penicillin, ampicillin, amoxicillin, cefuroxime, cefazolin, sulfamethoxazole, doxycycline, oxytetracycline, tetracycline, gentamicin, vancomycin, clindamycin, oxacillin, bacitracin, neomycin and streptomycin. The antibiogram discs manufactured by Conda, Proanalysis, were used. To control the performance of this method, we used the reference strain Staphylococcus aureus WDCM 00034. Using a disk diffusion test, 100% resistance of the isolates of *Staphylococcus pseudintermedius* was determined on ampicillin, cefazolin, clindamycin, bacitracin, neomycin and streptomycin. All *Staphylococcus pseudintermedius* isolates were multiresistant to six or more antibacterial drugs. *Staphylococcus pseudintermedius* isolates showed no resistance to vancomycin and doxycycline.

Keywords: *staphylococcus, resistance, antibacterial drugs*

Introduction

The *Staphylococcus* genus belongs to bacteria that have been studied for a long time but they still represent a big unknown. *Staphylococci* are found in humans and many animal species and also they can be found in foods of different origins and in the environment. Most *Staphylococcus* species are commensals found within the microbiome in humans and animals and do not lead to disease, but they are significant because they are often a reservoir of antibacterial drug resistance and virulence genes so although they do not harm the host, they can be a source of genes for other bacteria. Most of *staphylococci* have ability to transmit from one animal species to another, from animals to humans and inversely, although there are some species that are specifically adapted to specific host species or their body parts.

Until the mid-1970s, members of the *Staphylococcus* genus were divided into three species: coagulase positive *Staphylococcus aureus* and coagulase negative *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* (Gotz *et al.*,2006; Hill, 1981; Becker *et al.*,2014). The *staphylococci* ability to coagulate rabbit plasma has divided them into pathogens and non-pathogens (Salle, 1967), which today is losing significance because coagulase negative species of this genus have also been identified as pathogens (Smeltzer and Beenken, 2013). The coagulase positive species are: *S. aureus*, *S. intermedius*, *S. pseudintermedius*, *S. delfini*, *S. schleiferi* and *S. hyicus*. (Becker *et al.*,2014).

Using molecular diagnostics in samples from dogs, cats, horses, and parrot's, *S. pseudintermedius* was described in 2005, when it was suggested that isolates originating from dogs, unless molecular methods were available to identify such isolates, be attributed to this species (Bond and Loeffler, 2012). It is a conditionally pathogenic bacteria, part of the physiological microflora of the skin and mucous membranes of healthy dogs and cats and the most common cause of superficial and deep pyoderma, inflammation of the middle ear, pharynx, nose, eye, postoperative wounds in dogs and cats and other tissues (Futagawa-Saito *et al.,* 2004; Quinn *et al.,* 2011; Van Duijkeren *et al.,* 2011; Vericelli, 2009) but also a cause of sporadic infections in other species, including humans (Van Duijkeren *et al.,*. 2011; Weese and Van Duijkeren, 2010). Evidence of zoonotic transmission of *S. pseudintermedius* has been reported from dogs to humans (Paul *et al.,* 2011; Laarhoven *et al.,* 2011). Although information on the pathogenic effect of *S. pseudintermedius* is limited, is known that possess virulence factors similar to those found in *S. aureus* (Van Duijkeren *et al.,* 2011). Methyl resistant strains have been discovered in recent years (*S. pseudintermedius*-MRSP-resistant methicillin), and the incidence of isolation from healthy and sick dogs is increasing.

Bacteria have developed a resistance mechanism to all groups of antibacterial drugs that are used systematically in the treatment of certain diseases. The most common resistance mechanism involves changing the target site on cell, inactivation of an antibacterial drug by enzyme formation, decreased cell membrane permeability for drug entry or actively ejecting the drug from the cell (Opal, 2005). Methicillin resistance of *staphylococci* is not mediated by an enzyme, but by a changing target site in the cell membrane of the bacterium where antimicrobial drug is bound. Methicillin-resistant *S. pseudintermedius* strains are most commonly resistant to many antimicrobials from other groups of antibiotics, and one of the most common reasons is the frequent use of broad-spectrum antibacterial drugs, insufficient long-term treatment and inadequate dosage of the drug.

The transition of *staphylococci* from one host species to another allows the acquisition of new genetic material, often located on mobile genetic elements (Lowder *et al.,* 2009). Although mobile genetic elements make up only a quarter of the staphylococcus genome, they encode many virulence factors and antimicrobial resistance, which is why they play an important role in bacterial adaptation and survival (Malachowa and DeLeo, 2010; Lindsay and Holden, 2004). Methicillin-resistant *S. pseudintermedius* strains have been isolated from healthy and sick dogs, cats and humans and more cases of transmission of MRSA strains between humans and dogs have been reported (Steen, 2011; Paul *et al.,* 2011; Van Duijkeren *et al.,* 2004; Rutland *et al.,* 2009) and also between humans and cats (Weese *et al.,* 2006).

Bacteria adapt quickly to the environment thanks to rapid generation change (20 minutes on average), and following the laws of evolution. The mechanisms of resistance among different bacterial species are numerous, and there is no antibacterial drug that has not developed resistance. Rapid and accurate diagnosis is key to reducing unnecessary use of antibacterial drugs. The development of rapid microbiological diagnostics is important not only in the diagnosis of life-threatening infections, but also in outpatient treatment, in which timely treatmen guidance will substantially rationalize application of antibacterial drugs.

Antibacterial substances have different effects on certain types of microorganisms and they can cause cell death due to bacterial destruction or prevent their reproduction, so we can divide them into two groups based on this criterion:

1. Antibacterial drugs exhibiting bacteriostatic activity (tetracyclines, chloramphenicol, macrolides and sulfonamides)
2. Antibacterial drugs exhibiting bactericidal action (aminoglycosides, penicillins and cephalosporins).

The aim of this study was to examine the resistance of bacterial isolates of *Staphylococcus pseudintermedius* to penicillin, ampicillin, amoxicillin, cefuroxime, cefazolin, sulfamethoxazole, doxycycline, oxytetracycline, tetracycline, gentamicin, vancomycin, clindamycin, oxacillin, bacitracin, neomycin and streptomycin, using a disk diffusion method.

Materials and methods

Eleven swabs originating from 10 dogs and 1 rabbit were submitted to the Public Institution Veterinary Institute of the Republic of Srpska "Dr Vaso Butozan" Banja Luka (6 ear swabs, 4 skin swabs and 1 nose swab), for microbiological testing for the presence of bacteria and susceptibility testing isolates for antibacterial drugs in order to treat them as successfully as possible. Samples were delivered from a veterinary ambulance from the area of city of Banja Luka. (Entity of Repuublic of Srpska, Bosnia and Herzegovina).

The delivered samples were seeded on blood agar (blood agar base with 5% defibrinated sterile sheep blood) and nutrient agar and incubated aerobically for 24 hours at 37°C. *Staphylococcus pseudintermedius* was identified in all 11 samples by conventional microbiological methods.

The sensitivity of the isolated strains to antimicrobial drugs was performed by disc diffusion method on Mueller-Hinton agar, by direct suspension of colonies at a concentration 0.5 McFarland, according to the CLSI standard (Clinical and Laboratory Standards Institute) for the following antimicrobials: penicillin (P, 10IU), ampicillin (A, 10μg), amoxicillin (AX25μg), cefuroxime (CXM30μg), cefazolin (KZ, 30μg), sulfamethoxazole (SMZ, 50μg), doxycycline (DXT30μg) oxycitertoxicillert (OT,30μg), tetracycline (T, 30μg) gentamicin (CN, 10μg), vancomycin (VA, 30μg), clindamycin (DA, 10μg), oxacillin (OX, 1μg), bacitracin (B, 0,04μg), neomycin (N, 30μg), streptomycin (S, 10μg) (Conda, Proanalysis).

Staphylococcus aureus strain WDCM 00034 was used to control the process.

Growth zone inhibitions were interpreted as sensitive, moderately sensitive and resistant, as recommended by CLSI 2008 and CLSI 2014.

Results and discussion

The test results of 11 isolates of *Staphylococcus pseudintermedius* are shown in Table 1.

Table 1. Results of antimicrobials resistance of *S. pseudintermedius*

No.	Antimicrobial drug	Sensitive		Moderately sensitive		Resistant	
		n	%	n	%	N	%
1	Penicillin	5	45.45	0	0.00	6	54.54
2	Ampicillin	0	0.00	0	0.00	11	100
3	Amoxicillin	6	54.54	0	0.00	5	45.45
4	Cefuroxime	2	18.18	0	0.00	9	81.81

5	Cefazolin	0	0.00	0	0.00	11	100
6	Sulfamethoxazole	0	0.00	0	0.00	11	100
7	Doxycycline	11	100	0	0.00	0	0.00
8	Oxytetracycline	10	90.90	0	0.00	1	9.09
9	Tetracycline	10	90.90	0	0.00	1	9.09
10	Gentamicin	10	90.90	0	0.00	1	9.09
11	Vancomycin	11	100	0	0.00	0	0.00
12	Clindamycin	0	0.00	0	0.00	11	100
13	Oxacillin	8	72.72	0	0.00	3	27.27
14	Bacitracin	0	0.00	0	0.00	11	100
15	Neomycin	0	0.00	0	0.00	11	100
16	Streptomycin	0	0.00	0	0.00	11	100

Using disc diffusion method, complete resistance to ampicillin, cefazolin, sulfamethoxazole, clindamycin, bacitracin, neomycin and streptomycin was determined in all 11 (100%) isolates of *Staphylococcus pseudintermedius*, in 9 (81.81%) isolates on cefuroxime, in 6 (54.54%) on penicillin, in 5 (45.45%) on amoxicillin, in 3 (27.27%) on oxacillin and in one (9, 09%) on tetracycline, oxytetracycline and gentamicin. The isolates showed no resistance to vancomycin and doxycycline.

In relation to the degree of resistance to antimicrobial drugs, the resistance of *Staphylococcus pseudintermedius* is shown in Figure 1.

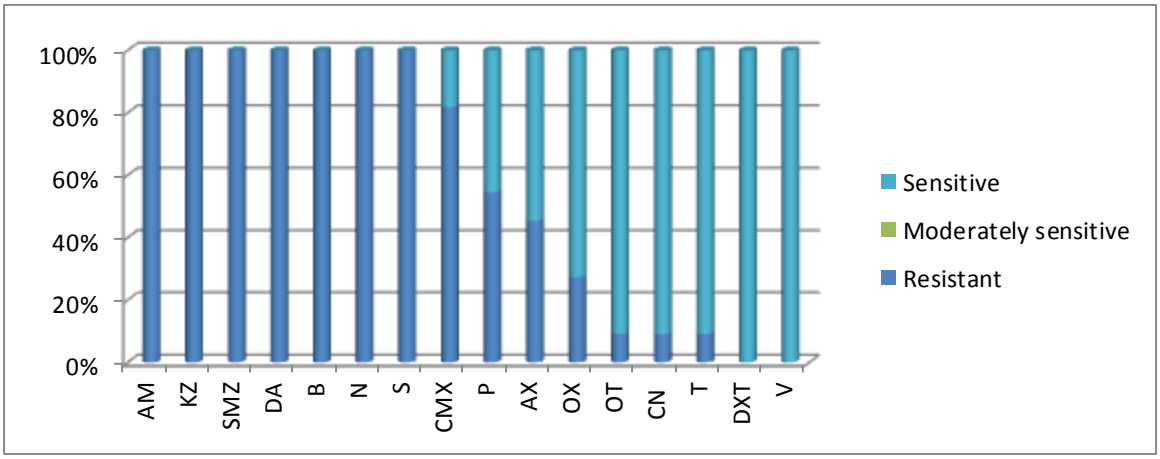


Figure 1. Resistance of Staphylococcus pseudintermedius to antibacterial drugs in relation to the degree of resistance

Staphylococcus pseudintermedius isolates are most commonly resistant to penicillin, ampicillin, tetracycline, erythromycin, linkomycin, and sulfamethoxazole with trimethoprim, to some extent, they are consistent with literature data (Holm *et al.*, 2002; Boerlin *et al.*, 2001; Vercelli, 2009). Staphylococcus pseudintermedius was previously thought to be susceptible to β-lactam antibacterial drugs, however, methicillin-resistant isolates, which are isolated from sick but also healthy dogs, cats and even humans, are increasingly emerging (Weese and Van Duijkeren, 2010).

In the analyzed strains of *Staphylococcus pseudintermedius*, 27.27% of the isolates were resistant to oxacillin, which is in line with the results of other studies (Vanni *et al.*, 2009; Meucci *et al.*, 2010; De Lucia *et al.*, 2011). Oxacillin-resistant strains probably belong to the methicillin-resistant strain of *Staphylococcus pseudintermedius*, which means that their target site for which β-lactam antibacterials binds has been changed and should be declared resistant to all β-lactam antibacterial drugs (all penicillins, cephalosporins, and imipenem), whether or not disc diffusion testing may have shown sensitivity to individual β-lactams (CLSI, 2014).

All isolates were streptomycin and neomycin resistant. Aminoglycoside antibacterial drugs act on a bacterial cell by binding to one or more places on the ribosome leading to inhibition of protein synthesis. To reduce the possibility of resistance to this class of antibacterial drugs in the treatment of staphylococcal infections, these drugs should be given in combination with other antibacterial drugs (Schito, 2006). If the isolate is gentamicin resistant, then resistance to all aminoglycosides is reported (Leclercq *et al.*, 2013).

The resistance of *Staphylococcus pseudintermedius* strains to amoxicillin (45.45%), which we determined, differs from the results of other studies (Yoon et al., 2010).

The major support for the treatment of MRSA-induced infections is the use of glycopeptides, most commonly vancomycin, to which none of *Staphylococcus pseudintermedius* isolates has been resistant.

The resistance of isolates of *Staphylococcus pseudintermedius* differs between studies. The reason for certain differences in results may also be the inadequate identification within the interpreted categories (diameter of inhibition zone) for particular antibacterial drugs, which are different for certain types of staphylococci. By 2005, the CLSI differentiate Staphylococcus *aureus* and coagulase-negative staphylococcus (CoNS) in their standards for the examination of staphylococcus susceptibility to oxacillin and cefoxitin, and after that along with *Staphylococcus aureus* also found *Staphylococcus lugdunensis* (CLSI, 2005). In 2013, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) also added *Staphylococcus saprophyticus* to the group *Staphylococcus aureus* and *Staphylococcus lugdunensis*, oposit other CoNS when it comes to testing the susceptibility of staphylococci to cefoxitin (EUCAST, 2013), and in 2014 added another group containing *Staphylococcus pseudintermedius* (EUCAST, 2014).

The values of the diameter of the inhibition zone differ significantly between the above groups, indicating the need for accurate identification in order to avoid errors in the interpretation of the results of antibiotic *susceptibility testing (AST)*. Also one of the reasons for the different results may be the composition of the medium, the pH of the medium, the temperature of incubation, as these all can affect the passage of bacteria through the medium.

Methicillin-resistant CoNS strains and methicillin-resistant *Staphylococcus pseudintermedius* strains possess similar resistance genes and they are also resistant to the same groups of antibacterial drugs (fluoroquinolones, macrolides, lincosamides and aminoglycosides), indicating their selection by the frequent use of antibacterial drugs in the treatment of animals, because these groups of antibacterial drugs, and in particular β-lactams and fluoroquinolones, are most commonly used in veterinary practice.

Bacterial resistance to antimicrobial drugs is certainly one of the leading problems of the 21st century. Bacteria have developed mechanisms of resistance to all groups of antibacterial drugs used in medicine. The occurrence of resistance depends on length of the using

antibacterial drug and the length of therapy. Prolonged use of low-dose antibacterial drugs represents the optimal selective pressure for resistance to bacteria (Levy, 2001). Also, overuse of antibacterial drugs as a growth promoter (Schito, 2006), as well as misuse due to inadequate choice of therapy (Fischer *et al.*, 2004) are the reason for the occurrence of resistant bacteria.

Of concern in this study is the occurrence of multiresistance in all the isolates tested. All 11 (100%) isolates of *Staphylococcus pseudintermedius* were multiresistant to 6 or more antibacterial drugs used.

Conclusion

Based on the results obtained by disc diffusion method, 100% of *Staphylococcus pseudintermedius* isolates showed complete resistance to ampicillin, cefazolin, sulfamethoxazole, clindamycin, bacitracin, neomycin and streptomycin, 54.54% of the isolates were resistant to penicillin, 45.45% to amoxicillin, 27.27 to oxacillin and 9.09% to tetracycline, oxytetracycline and gentamicin. All *Staphylococcus pseudintermedius* isolates (100%) were multiresistant to 6 or more antibacterial drugs. *Staphylococcus pseudintermedius* isolates showed no resistance to vancomycin and doxycycline. In conclusion, it should be point out that, wherever possible, all infections should be treated on the basis of laboratory tests and antibiotic *susceptibility testing*, especially in the case of recurrent infections. Also, non-antibacterial therapy should be used whenever is possible. In the fight against the spread of resistance to antibacterial drugs, scientific research is also playing an important role in discovering new mechanisms of resistance and the possibilities of overcoming and controlling it.

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ANALYSIS OF THE MICROBIOLOGICAL SAFETY OF FEED IN THE PERIOD 2017-2019 IN REPUBLIC OF SRPSKA (BOSNIA AND HERZEGOVINA)

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Abstract

The health safety of feed includes the appropriate chemical composition, sensory characteristics and microbiological safety of feed. The microbiological safety of feed directly affects the health status of animals and their production results, as well as the safety of food of animal origin. The aim of the study was to determine the microbiological safety of feed in the period 2017-2019 in Republic of Srpska (Bosnia and Herzegovina), during which 1,153 samples were tested. Samples were tested for *Salmonella* spp., coagulase positive staphylococci and *Staphylococcus aureus*, *Clostridium perfringens*, total number of microorganisms and total number of yeasts and molds, by test methods BAS EN ISO 6579, BAS EN ISO 6888-1, BAS EN ISO 7937, BAS EN ISO 4833-1 and BAS ISO 21527-2. Samples of feed were tested within self-control (82.15%) and official controls (17.85%). In relation to the Rulebook on microbiological criteria in feed, an average of 26.33% of unsatisfactory samples were identified, of which 97.08% were from self-control and 2.92% from official controls. On average, the highest percentage of unsatisfactory samples of feed was due to the increase in total number of microorganisms (18.86%) and increase in total number of yeasts and molds (16.95%), while the negligible percentage of unsatisfactory samples was due to the presence of pathogens *Salmonella* spp. (0.39%), *Clostridium perfringens* (0.30%) and coagulase-positive staphylococci and *Staphylococcus aureus* (0.08%).

Keywords*: feed, mirobiological criteria, safety, Bosnia and Herzegovina*

Introduction

The health safety of feed includes the appropriate chemical composition, sensory characteristics and microbiological safety of feed. The microbiological safety of feed directly affects the health status of animals and their production results, as well as the safety of food of animal origin.

Ultimate goal with regard to microbial load in feed is not giving sterile feed but to give feed with safe contamination level. Evaluation of feed safety for microbiological contamination and establishing a safer level for allowing entry into feeding is needed (Gopi *et al.*, 2017).

Microorganisms can be one of the causes of spoilage of feed, whether it is saprophytic microflora or subsequent contamination. Saprophytic bacteria in feed, expressed as a number of microorganisms, are regularly present in feed. Their presence should not be neglected since bacteria use nutrients from mixtures for their own metabolic functions, reducing their nutritional value and changing their organoleptic properties. It is obvious that they indirectly affect the quality of feed, so their presence in feed should be viewed in a broader aspect, taking into account a certain tolerance of their number above the allowable and the simultaneous presence of pathogenic bacteria.

When it comes to feed, contamination with yeasts and molds can occur at all stages in the food chain, it can occur before harvest and during storage. Contamination by yeasts,

especially molds, carries with it, in addition to changes in the appearance, taste and quality of food, the danger of contamination with mycotoxins. Mycotoxins are products of several types of toxogenic molds. Conditions for the development of mold are not always conditions for the production of mycotoxins, but an increased number of molds is always an indication of the possible presence of mycotoxins. High temperatures and dry periods are conducive to the reproduction of molds and the formation of mycotoxins. In recent years, on the global level, there have been significant climate disturbances, accompanied by extremely high or low temperatures, the appearance of large and extensive rains with floods and major droughts. All these influences have contributed to the increase of contamination by yeasts and molds, primarily cereals, but also other nutrients (Tournas, 2005; Moos, 2008; Barth, 2009). Excessive number of molds in feed mixtures for young animals was 43.5-48.1%, and in feed mixtures for older animals 14.1-17.2% (Mašić *et al.*, 2002). In the examination of samples of food for domestic animals and pets, the presence of fungi was not detected in only 12.2% of samples (Kubizna *et al.*, 2011). However, determining the number of fungi is not enough to assess the quality of feed mixtures, but for this it is necessary to determine the concentration of mycotoxins.

Nedeljkovic *et al.* (1994) observed a significant increase, not only in the number of contaminated feed samples, but also in the number of unsatisfactory samples, especially piglet feed mixtures, which are associated with climatic conditions. Given that they are mainly isolated genera of molds that contaminate nutrients and mixtures in warehouses, it is clear that such a large number of unsatisfactory mixtures is caused by errors made during storage and handling, both nutrients as components of mixtures and finished mixtures, as well as poor ambient conditions in which they are stored (Leeson *et al.*, 1995).

By far, the most important bacterial pathogen in feed is *Salmonella* which frequently occurs in a large number of feed ingredients of animal or plant origin and also in compounded feed. Other pathogenic bacteria with relevance for animal and human health and where feed might be a vector for the dissemination of the pathogen is limited to a few other species such is *Listeria monocytogenes, Escherichia coli* O157:H7 and *Clostridium* spp. (EFSA, 2008). *Salmonella* has been the pathogens of greatest focus and concern with respect to animal feed due to the organisms' ability to infect food producing animals and thereby pose a potential risk in human foodborne disease (Ge *et al.*, 2013; Jackson *et al.*, 2013; Molla *et al.*, 2010). *Salmonella* spp., one of the most important bacterial zoonotic agents, is an essential bacterium in the assessment of microbiological quality of feed (EFSA, 2006). However, comparing the results from the years 2009 – 2012 to the years 2003 – 2010, a slight decline in the number of samples contaminated with *Salmonella* spp. is permanently noticeable (Kwiatek *et al.*, 2008; Kukier and Kwiatek, 2011).

Presence of sulphite reducing clostridia in analyzed samples is an indication of rare, but fairly continious contamination of animal feed whit sulphite reducing clostridia (Jakić-Dimić *et al.*, 1994; Nedeljković *et al.*, 1994). Most of the tested mixtures for pig contained the permitted number of clostridia (97%), and in a small number of samples, mostly piglets, an impermissible number of clostridia was found, which ranged up to 1,700 CFU/g (Mašić *et al.*, 2002). Presence of sulphite reducing clostridia is not necessarily etiologically related to health disorders, and feeds in which their presence is not detected can contain their toxins. Therefore, the presence of sulphite reducing clostridia can be considered to be etiologically related to health disorders only when their numb is verified by confirmation of toxin-producing bacteria, i.e. toxin in the feed.

The aim of the study was to determine the microbiological safety of feed in the period 2017-2019 in Republic of Srpska (Bosnia and Herzegovina).

Material and Methods

The test material was 1.153 samples of feed, originating from the territory of the Republic of Srpska (Bosnia and Herzegovina). The survey was conducted in the period 2017-2019 in the Public Institution Veterinary Institute of the Republic of Srpska "Dr Vaso Butozan" Banja Luka (Bosnia and Herzegovina). Samples were submitted as a part of self-control and official control. In 2017, 442 samples were tested, in 2018, 374 samples, and in 2019, 337 samples of feed were tested. In relation to the method of sample delivery, the structure of samples is shown in Table 1.

Table 1. Structure of samples in relation to the method of delivery

Year	Self-control %	Official control %
2017.	89.37	10.63
2018.	82.89	17.11
2019.	74.18	25.82
$\bar{X} \pm \delta$	82.15 ± 7.62	17.85 ± 7.62

The following standard test methods were used for microbiological testing samples of feed:

1. BAS EN ISO 6888-1 (ISBIH, 2005a) for the enumeration of coagulase-positive staphylococci, *Staphylococcus aureus* and other species,
2. BAS EN ISO 7937 (ISBIH, 2005b) for enumeration of *Clostridium perfringens*,
3. BAS EN ISO 6579 (ISBIH, 2005c) for the detection of *Salmonella* spp.,
4. BAS ISO 21527-2 (ISBIH, 2009) for the enumeration of yeasts and moulds,
5. BAS EN ISO 4833-1 (ISBIH, 2014) for the enumeration of microorganisms.

In our research and in the statistical analysis of the obtained results, we used, as basic statistical methods, descriptive statistical parameters.

Results and Discussion

Results of microbiological testing of feed in the period 2017-2019 are shown in Table 2.

Table 2. Results of microbiological testing of feed

Year	Number of samples	Satisfactory samples %	Unsatisfactory samples %
2017.	442	70.59	29.41
2018.	374	75.94	24.06
2019.	337	74.48	25.52
$\bar{X} \pm \delta$		73.67 ± 2.77	26.33 ± 2.77

In the period 2017-2019, the percentage of feed samples that met the microbiological criteria averaged 73.67% and unsatisfactory 26.33%. Compared to the results of Golić and Nedić (2017), there is a significant improvement in the microbiological status of animal feed compared to the period 2014-2016, with the percentage of unsatisfactory samples lower by about 7%. In relation to the method of sample delivery, the structure of unsatisfactory samples of feed in the period 2017-2019 is shown in Table 3.

Table 3. Structure of unsatisfactory samples of feed in relation to the method of delivery

Year	Self-control %	Official control %
2017.	94.62	5.38
2018.	97.78	2.22
2019.	98.84	1.16
$\overline{X} \pm \delta$	97.08 ± 2.20	2.92 ± 2.20

Observing the structure of unsatisfactory samples of feed in relation to the method of delivery, it can be concluded that the self-control plans are adequate and that the awareness of producers regarding the production of safe animal feed is very developed.

Results of microbiological testing of feed in the period 2017-2019, according to the examined parameters from the Regulation on microbiological criteria in feed (Official Gazette B&H, 2012), are shown in Table 4.

Table 4. Results of microbiological testing of feed according to the examined parameters

Type of testing	Satisfactory samples %				Unsatisfactory samples %			
	2017.	2018.	2019.	$\overline{X} \pm \delta$	2017.	2018.	2019.	$\overline{X} \pm \delta$
Salmonella spp.	99.10	99.73	100	99.61±0.46	0.90	0.27	0	0.39±0.46
Coagulase positive staphylococci and *Staphylococcus aureus*	99.77	100	100	99.92±0.13	0.23	0	0	0.08±0.13
Clostridium perfringens	99.10	100	100	99.70±0.52	0.90	0	0	0.30±0.52
Number of microorganisms	80.32	84.76	78.34	81.14±3.29	19.68	15.24	21.66	18.86±3.29
Number of yeasts and molds	79.41	83.69	86.05	83.05±3.37	20.59	16.31	13.95	16.95±3.37

In the period 2017-2019, the percentage of feed samples that met the microbiological criteria for *Salmonella* spp. was 99.61% on average and 0.39% unsatisfactory on average. The obtained results are in accordance with the results of other authors. In the examination of components for feed for the period 2007-2010, the percentage of samples in which it is isolated *Salmonella* spp. ranged from 1 to 3.6%, average 2.15% (Kukier and Kwiatek, 2011), and in the study of complete feed mixtures for poultry, pigs and cattle for the period 2007-2010, the percentage of samples in which it is isolated *Salmonella* spp. ranged from 0 to 3.5% (Kukier et al., 2012). From current literature, the apparent prevalence of *Salmonella* (0.3-1.0%) in finished animal feed in New Zealand is similar to recent prevalence figures reported internationally (Cressey *et al.*, 2011). Golić and Nedić (2017) found 0.74% of unsatisfactory feed samples due to the presence of *Salmonella* in the period 2014-2016. In the period 2009-2012, in Poland, the percentage of feed materials contamination by *Salmonella* spp. ranged from 0.84% to 3.58% with an average value of 1.83% (Kukier *et al.*, 2013). The industry based data from 2005 and 2006 reports an incidence between 0 and 0.8% of *Salmonella* contaminated samples in compounded feed to different food animal species (poultry, swine and cattle) (EFSA, 2008).

In the period 2017-2019, the percentage of feed samples that met the microbiological criteria for coagulase-positive staphylococci and *Staphylococcus aureus* averaged 99.92%, and unsatisfactory averaged 0.08%. When it comes to coagulase-positive staphylococci in feed, there is not much data in the available literature. EFSA (2008) does not consider the pathogenicity of coagulase-positive staphylococci present in feed at all. Golić and Nedić (2017) did not determine the presence of coagulase-positive staphylococci and

Staphylococcus aureus in feed samples in the period 2014-2016. In connection with this, the question of the justification of the obligatory laboratory testing of feed on this parameter is raised. A special argument in support of the doubt about the justification of mandatory testing for this parameter, which should be taken into account, is the scientifically based, generally accepted fact, at which values of coagulase-positive staphylococci will be performed production of the enterotoxin ($>10^5$CFU/g). Certainly, in cases of suspected animal poisoning, testing of feed samples should be done in the presence of staphylococcal enterotoxins.

In the period 2017-2019, the percentage of feed samples that met the microbiological criteria for *Clostridium perfringens* was on average 99.70%, and unsatisfactory on average 0.30% (Golić and Nedić, 2017). The obtained results are in accordance with the results of Golić and Nedić (2017), who found 0.28% of unsatisfactory feed samples due to the presence of *Clostridium perfringens* in the period 2014-2016. In view of the common isolation of *Clostridium perfringens* from the environment and from the intestinal tracts of livestock (75% to 95% of broilers) (van Immerseel, 2004), and the fact that *Clostridium perfringens*-associated diseases appear to need initiators in addition to the presence of the organism (Songer, 1996; Craven, 2000; van Immerseel, 2004), the significance of feed contamination by this bacterium is open to question.

The percentage of feed samples that did not meet the microbiological criteria in the period 2017-2019 averaged 18.86% for the number of microorganisms, and for the number of yeasts and molds 16.95%. In terms of the number of microorganisms and yeasts and molds, the obtained results are in accordance with the research conducted by Čabarkapa *et al.* (2009), in which the cause of microbiological malfunction of feed was an increased number of microorganisms and an increased number of yeasts and molds. The same authors did not determine the presence of pathogenic microorganisms. Compared to the results of Golić and Nedić (2017), there is a significant improvement in the microbiological status of feed compared to the period 2014-2016, with the percentage of unsatisfactory samples due to the number of microorganisms lower by about 9%, and the number of yeasts and molds by about 5%. Proper judgment of the determined number of microorganisms in the examined samples is significantly hampered by unclearly formulated provisions of the rulebook regarding the definition of young and adult animals, having in mind the diversity of production categories of different animal species (Mašić *et al.*, 2002). In addition, the risk of mold in mixtures is related to the possible presence of secondary metabolites (mycotoxins), which are formed under certain conditions, so that judging the usability of feed only on the basis of number and type is incomplete (Leeson *et al.*, 1995).

It is desirable to include all feedstuffs for food animals into the microbiological risk assessment for feed. However, a risk based approach support a limitation of this report primarily to industrial compound feed. In addition too little is known about forage and home-grown cereals. Thus, all considerations of this report are focussed on the industrial compounded feed including the major risk ingredients in industrial compound feed: the protein rich vegetable protein and animal derived protein (EFSA, 2008). When it comes to limit values, the question is whether they are justified or whether they are set correctly in the current regulations, especially in the case of the number of microorganisms and the number of yeasts and molds, given that most samples are unsatisfactory precisely because of these parameters. We believe that the answer to this question and the question of a large number of unsatisfactory samples could be obtained by defining different categories of feed, by analogy with microbiological criteria for food, in accordance with the specifics of the technological procedure, for each category of feed. We remind that the Rulebook on microbiological criteria in feed (Official Gazette B&H, 2012) defines only five categories of feed (feed of plant and animal origin, feed mixtures for young and adult animals and pelleted mixtures).

Conclusion

In the period 2017-2019, 26.33% of unsatisfactory samples were identified, of which 97.08% were from self-control and 2.92% from official controls. On average, the highest percentage of unsatisfactory samples of feed was due to the increase in total number of microorganisms (18.86%) and increase in total number of yeasts and molds (16.95%), while the negligible percentage of unsatisfactory samples was due to the presence of pathogens *Salmonella* spp. (0.39%), *Clostridium perfringens* (0.30%) and coagulase-positive staphylococci and *Staphylococcus aureus* (0.08%). The obtained test results indicate possible causes of the determined state of health safety of feed, such as poor quality or unfavorable microbiological status of components for feed production, unsatisfactory hygiene in the production process and inadequate legislation defining microbiological criteria in feed. It is necessary to define all possible categories of feed in the rulebook, and for each of them mandatory microorganisms and limit values.

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ADAPTIVE PRACTICES OF LIVESTOCK BREEDERS TO CLIMATE CHANGE IN MOROCCO'S ARID RANGELANDS

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Abstract

Climate change (CC) is a reality and a serious challenge in the pastoral ecosystem of the high plateaus of eastern Morocco, because since the end of the 70s, this area has experienced proven manifestations of CC such as a significant decrease in rainfall and an increase in droughts' frequency. Accordingly, small ruminant rearing, major livelihood, has become more vulnerable due to its overreliance on climatic conditions. This study aims to exam the adoption by livestock breeders of adaptation practices in the face of climate change, taking into account the differences in wealth status in terms of the size of the sheep flock held, as well as to determine the factors influencing their implementation. Data were analyzed using descriptive statistics, Kruskal-Wallis test and multiple linear regression. Almost half of the breeders belong to the group of low adopters of adaptive strategies toward CC. There are significant differences (Chi-square = 48.90, $p < 0.001$, df = 2) between breeders' categories in terms of total number of adaptation practices implemented and that these are due to a very significant dissimilarity between small herders on the one hand and large and medium breeders on the other. Regression results indicated a strong relationship between the total count of adaptation measures embraced and predictor variables ($F(8, 156) = 30.91$, $p < 0.001$, $R = 0.783$, $R^2 = 0.613$). The significant factors are: size of the sheep flock, equipment, ancillary activity, heavy rains perceived, temperature change perceived and agroecological site. It is therefore suggested to target small-scale breeders as a priority and provide an affordable equipment and improved climate information in future programs aimed at strengthening local-level adaptation to CC.

Keywords: *Climate change, Adaptation, Livestock breeders, Rangelands, Morocco*

Introduction

The high plateaus of eastern Morocco (HPEM) are one of the wider pastoral ecosystems in the country, covering a total landmass of about 35,000 km^2, which accounts for 10% of the total area of existing rangelands. From the end of the 1970s, this region has experienced proven manifestations of climate change. The rainfall decreased considerably (Fink et al., 2010; François et al., 2016; Melhaoui et al., 2018) whereas the temperature and the droughts' frequency have increased (Moroccan Meteorological Office, 2007, François et al., 2016; Melhaoui et al., 2018). Thus, a drier and warmer weather conditions have become more frequent (Born et al., 2008). In addition, small ruminant rearing, mainly sheep, is the major economic activity of households. However, this livestock raising of extensive type is particularly vulnerable toward CC as it depends largely on natural resources and therefore on climate conditions, which are characterized by increasingly low and erratic rainfall and recurrent droughts (Bechchari et al., 2014; Melhaoui et al., 2018). Poorest breeders with livestock rearing-based livelihoods are hardest hit due to their high vulnerability to the negative effects of CC (Maroc, 2011).

The main impacts of climate change on the study area are multiple and affect both the human and natural environment. They include the decline in rangeland productivity and increased water stress, thus causing a shortage of pastures and water resources (Mahyou et al., 2010; Maâtougui et al., 2011). This scarcity leads to even more competition or conflicts over the available natural resources (Bourbouze and El Aich, 2000). Other effects are also reported in the literature such as accentuation of the precariousness of the poorest households, of the rural exodus (El Harizi et al., 2005) and even of the social inequalities between pastoralists noted during episodes of prolonged droughts (Schilling et al., 2012). Climate change induced extreme events (droughts) are considered to be an amplifying factor in the degradation of rangelands at the study area, which has also been caused by overgrazing, plowing and anarchic cultivation of marginal areas and uncontrolled land clearing (Mahyou et al., 2010; Maâtougui et al., 2011; Schilling et al., 2012). Hence, in view of all these multiple consequences induced by climate change, the sustainability of pastoral livestock rearing seems to be seriously threatened.

To deal or overcome these negative impacts related to climate change, livestock breeders in the HPEM have undertaken a host of coping and adaptation responses. They include pastoral mobility, raising of mixed species herds, integrated crop-livestock farming, use of emigrants' remittances, commercializing of livestock, feed storage, destocking, wage laboring, using subsidized livestock feed, migration, diversification of livelihoods and insurance (Bourbouze, 2000; Bourbouze and El Aich, 2000; Schilling et al., 2012; Bechchari et al., 2014). However, these adaptation and mitigation options are for the most part low efficient and not very sustainable (Bourbouze, 2000; Bourbouze and El Aich, 2000). Furthermore, in order to support breeders to minimize or cope with the adverse impacts of climatic hazards, in particular drought, public policy has introduced some measures, the most important of which are the subsidization of livestock feed and the implementation of insurance against climatic risks. However, these public interventions, in particular financial incentives, have been unsuitable for pastoral lands and have given rise to negative side effects such as sedentarization and overgrazing (Schilling et al., 2012) as well as the clearing of pasture areas in order to convert them into cultivated lands. Note that the insurance mentioned above only covers crops and not livestock.

In this context, the study attempts to guide and assist livestock breeders and policy decision-makers to establishing effective, appropriate and sustainable adaptation responses in face to CC. In fact, the CC adaptation literature suggests that adaptation is conditioned by a host of factors (Ouédraogo et al., 2010; Below et al., 2012; Mabe et al., 2014; Tiwari et al., 2014; Berhanu and Beyene, 2015; Taruvinga et al., 2016). They include the socioeconomic attributes of farmers such as age, education and household size; institutional factors such as access to credit, extension services and the market; geographic factors or agroecological characteristics (location) as well as perception variables. Consequently, the identification of these target factors will help promote and strengthen CC adaptation at the local level. Hence, the objective of this study is to analyze the main adaptation practices used by the livestock breeders in adapting to climate change and to determine the key factors that influence their adoption (implementation).

Materials and Methods

Study area

The study was carried out in the high plateaus of eastern Morocco (HPEM), which lie in the 30S UTM zone. This region was purposefully chosen due to its high sensitivity to climate change and it can be considered as the most suitable area for in-depth understanding of CC adaptation practices in the pastoral ecosystems of Morocco. The climate is of Mediterranean

type but under important influence from the Sahara. Average annual rainfall varies between 143 mm in the southern part and 201 mm in the northern area (Melhaoui et al., 2018) and is highly variable, with coefficients of variation of about 45 and 34%, respectively. The mean annual temperature is 19 °C (Ben El Mostafa et al., 2001). Dry hot winds often lead to recurrent sandstorms during the summer season. Although raising small ruminants, mainly sheep, in natural pastures is the main livelihood of the local population, differences between three studied sites in terms of agricultural production systems practiced are observed. In the south agroecological site, extensive pastoral farming of small ruminants combined with localized rain-fed agriculture (cereal crops) is predominant. Given the low agricultural potential of this area and its high poverty rate, a significant part of local breeders, regularly engage in activities generating additional income such as occasional work and the services. Benefiting of their large pastoral lands, herders belonging the intermediate agroecological site mainly specialize in the extensive breeding of small ruminants and still maintain forms of traditional pastoralism such as habits of the nomadic way of life, social solidarity and pastoral agreements with other ethnic tribes. Regarding the north agroecological site, the small ruminant and bovine cattle rearing system is more intensive and it is integrated with a localized irrigated agriculture mainly of fodder crops. Thus, this area exhibits the high agricultural potential compared to the other sites.

Data collection

Data were gathered through a questionnaire survey conducted from September to December 2015 among 167 livestock breeders, heads of pastoral households. The questionnaire items cover the demographic and socioeconomic characteristics of the respondents, their perceptions toward climate change, the institutional and geographical factors such as access to credit, training and extension and the characteristics of the location of breeders interviewed, as well as the types of adaptation strategies used. Two-stage sampling was used to select breeders to be interviewed. The first stage involved the purposive selection of three study sites, namely the north agroecological site (rural territorial collectivity of Bni Mathar), the intermediate agroecological site (rural territorial collectivises of Tendrara, Maâtarka) and the south agroecological site (rural territorial collectivises of Bni Guil and Abbou Lakhal), based on a set of criteria (local climate type, extent of the available natural pastures and water resources) indicating differentiated agroecological contexts. Following this, in accordance with the local agricultural extension services, three categories of livestock breeders were identified, based upon the sheep herd size in ownership, which has been chosen as the discriminating criterion between breeders in the study area. Hence, large breeder is the one who owns a herd of more than 300 sheep, medium breeder has a sheep herd of between 101 and 300 heads and small-scale breeder with a sheep flock not exceeding 100 heads. Commensurately to the relative representativeness of these three breeders' categories in each selected site, the respondents were selected at random and distributed as follows: 96 small, 47 medium and 24 large breeders.

Data analysis

Data collected were analyzed using descriptive statistics, Kruskal-Wallis test and inferential statistical tool. The Kruskal-Wallis test was applied to highlight possible differences between the three identified categories of breeders in terms of the total count of CC adaptation practices implemented. Indeed, this test is an appropriate nonparametric analysis of variance test that assesses the differences between three or more independent samples compared to a single continuous variable that presents a non-normal distribution (McKight and Najab, 2010). Once the Kruskal-Wallis statistic is significant, multiple comparisons procedures are performed to identify categories of breeders that are different from the others. To ascertain the

major factors conditioning livestock breeders' adoption (implementation) of adaptive practices in the face of climate change in the study area, a multiple linear regression was used.

Empirical model and variables

To find out the important factors that influence farmers' adaptation against CC, different types of regression models were used in literature, depending on the type of the dependent variable (quantitative or qualitative) and its nature (continuous, binary, nominal or ordinal). For instance, the binary logit model is used when the response variable is a dichotomous variable (Mabe et al., 2014; Tiwari et al., 2014). But when the outcome variable is a quantitative continuous or counting variable, the multiple linear regression is then the most suitable form of regression analysis as have shown Yila and Resurreccion (2013), Arimi (2014) and Taruvinga et al. (2016).

The dependent variable in our analysis indicates the total count of climate change adaptation practices implemented (adopted) by each livestock breeder in the study area. Since its values result from counting, it is thus treated as a discrete quantitative variable. Therefore, the multiple regression model is the appropriate regression tool to explore the influence of a mixture of quantitative and qualitative independent variables (biophysical, socioeconomic, institutional and perception factors) on the dependent variable. The model is specified as follows (Eqn. 1):

$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots\ldots\ldots\ldots\ldots + \beta_n X_n + \mu$ (Eqn. 1)

Where Y is the dependent variable, β_0 is the Y- intercept, $\beta_1, \beta_2, \ldots, \beta_n$ are the coefficients of the independent variables to be estimated, μ is the error term whereas $X_1, X_2, \ldots, X_n$ are the explanatory variables hypothesized to affect breeders' adaptation practices. The predictors used in our model were identified based on literature and our knowledge of the study area. Table 1 recapitulates and describes the explanatory variables included in the regression model. The likely influence of these factors on the adaptation against CC at local level is given in the expected sign column. The degree to which these hypothesized factors affect the decision of breeders to adopt CC adaptation strategies differed as shown in the last subsection of results.

Table 1: Description of explanatory variables used in the regression model

Variables	Description	Type	Values/Measure	Expected sign
X_1	Age	Continuous	Years	±
X_2	Household size	Continuous	Numbers	+
X_3	Labor force	Continuous	Numbers	+
X_4	Ancillary activity of HH	Dummy	1: if practice and 0: Otherwise	±
X_5	Farm size	Continuous	Hectares	+
X_6	Sheep herd size	Continuous	Heads	+
X_7	Equipment	Continuous	Numbers	+
X_8	Access to formal credit	Dummy	1: if access and 0: Otherwise	+
X_9	Membership in CBO	Dummy	1: Yes and 0: Otherwise	+
X_{10}	Training	Dummy	1: Yes and 0: Otherwise	+
X_{11}	Veterinary care	Dummy	1: Yes and 0: Otherwise	+
X_{12}	Perceived frequent droughts	Dummy	1: if perceived and 0: Otherwise	+
X_{13}	Perceived heavy rains	Dummy	1: if perceived and 0: Otherwise	+
X_{14}	Perceived temperature change	Dummy	1: if perceived and 0: Otherwise	+
X_{15}	North agroecological site	Dummy	1: North site and 0: Otherwise	+
X_{16}	Intermediate agroecological site	Dummy	1: Intermediate site and 0: Otherwise	±

HH: Household head; CBO: Community-based organization

Results and discussion

Livestock breeders' adaptation against climate change

The Table 2 summarizes the adaptive practices implemented by the livestock producers in the study area against CC. The most common of them include the cultivation of cereal crops alongside the rearing of small ruminants, diversification of livestock portfolios composed of mixed flocks of sheep and goats, livestock feed storage, particularly barley, use insurance to protect against potential crop losses caused by extreme climate events, regular sale of part of the herd to buy livestock feed and pastoral mobility, particularly in the case of prolonged droughts. In addition, an important share of the breeders interviewed resort to non-farm coping strategies in order to diversify their sources of income, such as casual employment, conversion of part of the livestock capital into real estate speculation in urban areas, allowing a high return on investment and seeking of livestock feed on credit from resellers of such feeds. Previous literature reports similar adaptation practices (Bourbouze, 2000; Bourbouze and El Aich, 2000; Schilling et al., 2012; Bechchari et al., 2014; Berhanu and Beyene, 2015).

Table 2: Climate change adaptation practices used by livestock breeders in the HPEM (in %)

Adaptation practices	HPEM	Small	Medium	Large
Mixed agriculture-livestock system	83.2	74.0	93.6	100.0
Profit from Government support	79	75.0	83.0	87.5
Mixed-species rearing	77.8	71.9	85.1	87.5
Climate insurance	47.9	28.1	70.2	83.3
Livestock feed storage	47.9	36.5	63.8	62.5
Pastoral mobility	40.1	27.1	48.9	75.0
Frequent sale of livestock to buy animal feed	47.3	44.8	53.2	45.8
Sale of livestock in good physical condition	43.7	33.3	48.9	75.0
Integration of irrigated crops and livestock	13.2	8.3	21.3	16.7
Migration to urban areas	16.8	13.5	21.3	20.8
Paid casual work	32.9	42.7	27.7	4.2
Collection of truffles to acquire extra revenue	8.4	13.5	2.1	0.0
Rangelands appropriation for private use	18	13.5	17.0	37.5
Informal credit	22.2	18.8	19.1	41.7
Livestock fattening practice	30.5	20.8	31.9	66.7
Benefit of free support from friends and the tribe	13.2	14.6	8.5	16.7
Conversion of livestock capital into real estate investments	40.1	29.2	44.7	75.0

Furthermore, the adoption (implementation) of coping and adaptation measures in the face of climate change differs substantially across the breeders' categories. The large breeders adopt with higher frequency the most of available adaptation options and exhibit a rich adaptation portfolio diversity. Except for the off-farm practices which are more embraced by the small livestock keepers, such as casual labor and collection of truffles to acquire additional incomes. These coping practices are often used to overcome climate-induced risks and other insecurities sources. The differences found between breeders with regard to the number and nature of the adaptation measures undertaken, are mainly attributed to differences in wealth status, expressed in terms of the size of the livestock ownership. This finding is consistent with other studies (Deressa et al., 2009; Bechchari et al., 2014; Berhanu and Beyene, 2015; Taruvinga et a., 2016).

The results show that on average, the total count of adaptation strategies to climate change adopted per breeder is 7 (SD = 2.186). Indeed, the large breeders have the highest median

(and, hence, the mean of the total count of adaptive measures practiced) either 12 compared to small (6) and medium herders (8). In addition, almost half of the herders interviewed belong to the low adopter group (Figure 1). The Chi-square independence test was conducted to look at whether there is a link between breeders' categories (small, medium, large) and the adopter group, which is based on classes of total count of adaptation practices adopted. The relationship between these variables was highly significant, χ^2 (4, N = 167) = 49.54, $p < 0.001$ at the $\alpha = 0.05$ level of significance. In addition, examination of the Cramer's V. coefficient suggests that this association is relatively strong (V= 0.385, n = 167, $p < 0.001$). As a result, the distribution of the total number of adaptation strategies used differs considerably between categories of breeders. Indeed, large livestock owners are more likely to embrace more adaptation strategies in the face of CC and thus dominating the group of high adopters with 83%. Contrariwise, given that nearly 80% of small breeders were classified in the low adopter group, this testifies that their adaptation actions used in response to observed climate changes are poorly diversified.

The Kruskal-Wallis test was carried out to examine the differences found in the total number of adaptation practices adopted within the categories of breeders. The results reveal that there are significant differences (Chi-square = 48.90, $p < 0.001$, df = 2) among breeders' categories. In addition, the pairwise comparison shows that this lack of similarity among the categories of breeders is due to a very significant difference (Dunn's test, $p < 0.001$) between small herders on the one hand and large and medium livestock keepers on the other.

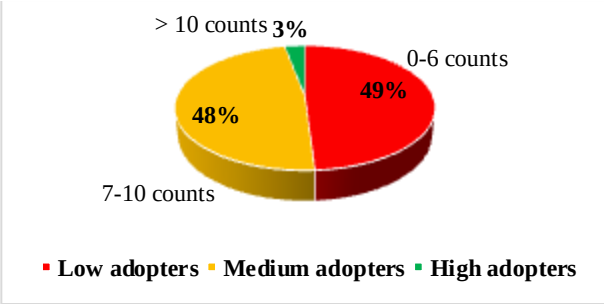


Figure 1: Classification of livestock breeders by total number of adaptation practices adopted

This finding is corroborated by Bechchari et al. (2014) who expressed that the ability for livestock owners in the study area to adapt in response to extreme climatic events (droughts) differed among them according to their respective socioeconomic status. They added that small-scale breeders have implemented least adaptation measures compared to large breeders who are slightly less affected and have a rich adaptation portfolio diversity. Similarly, the size of the herd of livestock in possession significantly and positively influences the decisions and choices of farmers (Balew et al., 2014) and pastoralists (Berhanu and Beyene, 2015; Opiyo et al., 2015) regarding the CC adaptation strategies to be implemented.

Determining factors for the adoption of adaptation measures in face to climate change

Using the enter method, results of the multiple linear regression indicate a strong relationship between the total count of adaptation measures adopted and predictor variables (F (8, 156) = 30.91, $p < 0.001$, R = 0.783, R^2 = 0.613). The model has a significantly high level of explanatory power as reflected in the R^2 value. Thus, used independent variables can explain a reasonable proportion of the variability of the response variable, that is 61 %. The R value indicates that the data fit well with the regression model. The F ratio of predictor variables in the model is statistically significant at 0.001. This shows that the variables used are suitable. Out of the 16 independent variables initially introduced into the model, only eight significantly explain the variation in the total number of CC adaptation strategies adopted within breeders (Table 3).

Table 3: Factors that influence the adoption of CC adaptation practices by the livestock breeders in the HPEM

Variables	Unstandardized coefficients β	Std Error	Standardized coefficients Beta	t-values	Sig.
(Constante)	4.847	0.462		10.486	0.000
Sheep herd size	0.004	0.001	0.415	5.790	0.000
Equipment	0.187	0.074	0.193	2.535	0.012
Ancillary activity	1.034	0.246	0.235	4.198	0.000
Practice of veterinary care	1.305	0.283	0.305	4.611	0.000
Perceived heavy rains	2.098	0.308	0.469	6.806	0.000
Perceived temperature change	-1.510	0.386	-0.211	-3.912	0.000
North agroecological site	1.394	0.405	0.254	3.444	0.001
Intermediate agroecological site	-1.327	0.342	-0.313	-3.878	0.000

Sheep herd size

A one standard deviation positive change in the size of sheep herd in ownership, holding other predictors constant, increases of 0.004 standard deviation for adopting of CC adaptation practices. Furthermore, the total count of adaptation practices adopted and the size of sheep flock were found to be strongly and positively correlated ($r = 0.59$, $n = 167$, $p < 0.001$). This indicates that the larger the size of the sheep herd in the breeder's possession, the greater the total number of CC adaptation measures that he implements. Hence, the size of livestock herd has a positive and significant effect on the likelihood of adopting climate change adaptation responses. This finding is corroborated by Balew et al. (2014), Bechchari et al. (2014), Obayelu et al. (2014), Berhanu and Beyene (2015) and Opiyo et al. (2015).

Possession of equipment

For a one standard deviation positive change in total number of heavy machines in ownership, holding other predictor variables constant, the results reveal an increase in the total count of CC adaptation adopted by 0.187 standard deviation. In addition, there is a significant relationship between the countable number of CC adaptation practices adopted by each breeder and the total number of equipment he owns ($r = 0.491$, $n = 167$, $p < 0.001$). Thereby, we can posit that the more the breeder/farmer is equipped with agricultural or transport equipment, the more he is likely to adopt or implement adaptive practices. Hassan and Nhemachena (2008) and Ouédraogo et al. (2010) expressed that the possession of heavy machinery was an important determinant influencing the African farmers' adoption of climate change adaptation methods.

Secondary occupation

The slope of the variable, namely ancillary activity of household head, is 1.034. This means that for every one unit increase in this factor, the total number of adaptive practices implemented increases by 1.034 standard deviation. The practice of a secondary activity such as occasional salaried work, commercial activity or public employment, in addition to the main profession (livestock breeding), allows some breeders to have additional incomes which could be used to adopt new adaptation methods in response to the observed climate changes. In line with this finding, many authors found that off-farm income increases the farmers' likelihood of undertaking CC adaptation responses (Deressa et al., 2009; Mabe et al., 2014; Obayelu et al., 2014). On the contrary, Tiwari et al. (2014) and Opiyo et al. (2015) indicated that this factor had non-significant effect on adaptation against climate change in rural farming in Nepal Himalaya and among Turkana pastoralists in northwestern Kenya, respectively.

Perception of long-term change in rainfall and temperature

The study reveals that the breeders' perception toward CC is another determining factor that influences the adoption of adaptation measures. This finding is consistent with other previous

studies (Debalke, 2011; Balew et al., 2014; Ndamani and Watanabe, 2016). For every one unit increase in the "heavy rains perceived" factor, the countable number of adaptive practices embraced increases by 2.098. Thus, the herders' who perceived a change in rainfall pattern over the last decades are more likely in adapting to CC. Similarly, Piya et al. (2012), Balew et al. (2014), Mabe et al. (2014) and Berhanu and Beyene (2015) have pointed out that farmers' perceptions toward long-term changes in precipitation significantly and positively influence their decisions and their choices of the adaptation actions to be implemented.

The coefficient of temperature' change perceived is negative. Indeed, for a one standard deviation positive change in this factor, holding other explanatory variables constant, the results indicate a decrease in the total count of adaptation practices adopted by 1.51. Thus, the observed results suggest that breeders' who perceived a change in temperature are less likely to adapt against climate change. Likewise, Debalke (2011) has found a negative effect of this factor on the adoption of some adaptation techniques such as soil conservation, irrigation and changing planting dates in the north shoa zone of Amhara region Ethiopia. In contrast, other studies have highlighted that the perception of temperature change positively and significantly affects the farmers' implementation of CC adaptive actions respectively in the Northern Ghana and Dera woreda, south Gondar zone, Ethiopia (Mabe et al., 2014; Atinkut and Mebrat, 2016).

Agroecological setting

As expected, breeders belonging diverse agroecological locations adapt differently to climate change. Indeed, differences were found between the three selected study sites in terms of the number and type of CC adaptation actions that were adopted (implemented) there. This can be explained mainly by contrasting biophysical conditions generating differentiated agroecological potentials. This finding is consistent with other studies (Ouédraogo et al., 2010; Below et al., 2012; Piya et al., 2013; Tiwari et al., 2014; Atinkut and Mebrat, 2016). A one standard deviation positive change in agroecological north site increases the total number of CC adaptive practices adopted by 1.394 standard deviation. Thus, livestock rearing in the north area, which exhibits the high agroecological potential, has a positive and significant impact on the probability of using more CC adaptation options, compared with raising in the two other locations. This finding is corroborated by Below et al. (2012) who underlined that the frequencies of adoption of CC adaptation strategies are significantly higher in the Mlali ward where the agroecological potential is much better compared to the Gairo ward. The distribution of the majority of adaptation actions differs considerably between the two settings as well.

Conclusions

The study revealed that there are significant differences within breeders in terms of the total count of CC adaptive measures embraced, mainly due to differences in the size of the herd held. Thus, the large livestock producers have undertaken a wide and varied range of adaptation actions compared to small-scale breeders which the majority belongs to the low adopter group since they exhibit a poor adaptation portfolio diversity. In addition, the significant factors that influence the adoption (implementation) of climate change adaptation practices at the household level in the study area, are of socioeconomic, geographic and perception type.

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CURRENT SITUATION OF CATTLE HUSBANDRY AND BREEDING POSSIBILITIES IN VAN PROVINCE OF EASTERN ANATOLIA IN TURKEY

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Abstract

The aim of this survey is to put forward the current situation and the potential of cattle husbandry and breeding opportunities in Van province of Eastern Anatolia in Turkey. Livestock activities in Van province of Eastern Anatolia are an important source of income for the local people. However, it can be said that the activities of animal husbandry have stagnated for last years. Even though this is the case, livestock activities and especially cattle breeding are indispensable for indigenous people. Van is one of the most prominent cities of this area in which its public sustain themselves by animal production. For this reason, it is very important to define the condition, potential, and problems of breeding sector especially with respect to the cattle stock in this city. With the inquiry works, results are determined as yield of cattle is low, the number of animals raised are small, possibility of marketing is restricted, breeding condition of animal is inconvenient, technical knowledge is insufficient, membership at the cooperative is incapable, veterinarian service is expensive. Lack of food, especially lack of protein is still basic problem in Turkey. Genetic improvement offers solutions for satisfaction of needs in livestock husbandry. In Van province of Eastern Anatolia, cattle husbandry participates with 2.5% in general livestock production in Turkey with high share. Characteristics of cattle husbandry in Van province are small farms with 2 to 3 cows per farm (80-85% of total number of farms). The most important of native cattle breeds of Turkey is the Eastern Anatolia Red breed. The cow milk from this breed is either consumed by the household as raw milk or processed as herbaceous cheese. As a result, preservation and development of native cattle breeds as a genetic source is very important.

Keywords: *Cattle breeding, Improvement, Traditional breeding system, Van province.*

Introduction

Cattle breeding in Anatolia has been adapted to regional differences and has been characterized by the prominence of different applications. With the inquiry works, results are determined as yield of cattle is low, the number of animals raised are small, possibility of marketing is restricted, breeding condition of animal is inconvenient, technical knowledge is insufficient, membership at the cooperative is incapable, veterinarian service is expensive. Lack of food, especially lack of protein is still basic problem in Turkey. Genetic improvement offers solutions for satisfaction of needs in livestock husbandry.

Livestock in Eastern Anatolia province are mostly carried out with traditional methods. Therefore, it will provide contribution to knowledge of the current problems of the industry in the province the current situation of the livestock sector and needs to be determined and the current situation to the steps taken to improve (Şeker and Köseman, 2015). The first practices that should be done to increase the profitability in livestock enterprises should be directed to environmental regulations. As the environmental factors improve, the genotype needs to be improved accordingly (Öztürk, 2009).

The most common diseases in cattle enterprises of Van province are foot and mouth disease, brucellosis, mastitis. Farmers are generally evaluating their milk themselves. Mostly, farmers are milking by hand.
The aim of this survey is to put forward the current situation and the potential of cattle husbandry and breeding opportunities in Van province of Eastern Anatolia in Turkey. Livestock activities in Van province of Eastern Anatolia are an important source of income for the local people. However, it can be said that the activities of animal husbandry have stagnated for last years. Even though this is the case, livestock activities and especially cattle breeding are indispensable for indigenous people. Van is one of the most prominent cities of this area in which its public sustain themselves by animal production. For this reason, it is very important to define the condition, potential, and problems of breeding sector especially with respect to the cattle stock in this city.

Demographic Structure of Van Province

Van province is located in Lake Van in Eastern Anatolia Region Chapter of the Upper Murat-Van in a closed basin on Turkey. The border with the Iranian State lies to the east of Van. Van is surrounded by Van Lake from west. It is also surrounded by cities such as Agri from north and Bitlis from west, Siirt, Şırnak, Hakkari from south and Iran from east. Van city is nineteenth the most crowded city in Turkey. As of the end of 2019, its population is 1.136.757 people. Van is the fifth largest province in terms of surface area in Turkey. Its area is 21.334 km^2. Although Van Lake and its valleys are covered with rich vegetation, the mountains are generally barren (Anonymous, 2020). Economy of Van province is an economy mainly based on agriculture and animal husbandry, aquaculture, and tourism. Small ruminant husbandry, cattle breeding, and especially the last poultry farming in recent years. Livestock sector in Van province every is developing day by day and gaining importance.
As Van province is in the lower status in terms of socio-economic development (in the 5th level), its unemployment rate high and employment rate low, and more than half of the working population in different sectors is concentrated on the agriculture sector significant migrations occurred in the province (Elmastaş and Yılmaz, 2015). The educational status of the cattle farmers and families of Van province is low. This will have a negative impact on the care and feeding of animals and the productivity of the products to be obtained. For this purpose, meetings where business owners can get technical information can be organized and small units can be created and information can be transferred from village to village. Thus, it will be contributed both to the conscious raising of animal husbandry and the economy of the country.

The Importance of Cattle Breeding in Van Province

Cattle husbandry is an industrial sector that they transform the natural vegetation cover pasture and the pasture not used in the agriculture into the products such as meat and milk. Cattle husbandry is indispensable and an important source of income for farmers in Van province. Van province is suitable for both small ruminant breeding and cattle husbandry in terms of large pasture areas, water resources, and climate characteristics.
The purpose of sheltering animals is to eliminate the negative effects of the environment on animals within economic limits and to provide comfortable living conditions suitable for their behavior. For this reason, when designing animal shelters, they should be dimensioned so as to provide sufficient space and internal detail for the movement, social, feed and water drinking behaviors of animals, and should be kept within economic and optimal limits in care management and hygienic conditions (Mutaf et al., 2001).

It can be said that the province is rich in terms of underground and surface irrigation sources as well as a suitable land structure for the production of forage crops. However, small ruminant husbandry is a major industrial sector in Van province in the Eastern Anatolia of Turkey (İnan and Aygün, 2018). Meanwhile, it is directly dependent on the involvement of the breeder to be successful in all studies to improve breeding and environmental factors at the breeder level.

Number of Cattle in Van Province

Characteristics of cattle husbandry in Van province are small farms with 2 to 3 cows per farm (80-85% of total number of farms). The most important of native cattle breeds of Turkey is the Eastern Anatolia Red breed. It is very important to define the condition, potential and problems of breeding sector especially with respect to the cattle, sheep, and goat stock in this city. Correspondingly, it could be possible to find short, average and long term solutions for the identified issues. The most important of these problems is roughage. Number of cattle according to genotypes in Turkey is presented in Table 1.

Table 1. Number of cattle according to genotypes in Turkey (head) (TOB, 2020).

Year	Cultural	%	Cross	%	Native	%	Total
2015	6385343	45.63	5733803	40.97	1874925	13.40	13994071
2016	6588527	46.8	5758336	40.9	1733292	12.3	14080155
2017	7804588	48.9	6536073	40.9	1602925	10.1	15943586
2018	8419204	49.40	7030297	41.25	1593005	9.35	17042506
2019	8762165	48.49	7640939	42.28	1667396	9.23	18070500

Inventory studies constitute the basis of all kinds of studies that are planned to be carried out at national or regional level. Therefore, important suggestions for the Van region will be presented with this study. Number of cattle and buffalo is presented in Table 2 in Turkey and in Table 3 in Van province.

Table 2. Number of cattle and buffalo in Turkey (head) (TOB, 2020).

Year	Cattle	Buffalo	Total
2015	13994071	133766	14127837
2016	14080155	142073	14222228
2017	15943586	161439	16105025
2018	17042506	178397	17220903
2019	18070500	180826	18251326

Table 3. Number of cattle and buffalo in Van province (head) (TOB, 2020).

Year	Cattle	Buffalo	Total
2015	166944	264	167208
2016	162172	308	162480
2017	185349	394	329688
2018	176438	536	176974
2019	179713	490	180203

The numbers of indigenous cattle are low and there is need for conservation and spread of indigenous pure breeds on other suitable areas. In Van province of Eastern Anatolia in Turkey, the indigenous cattle were found more in numbers. Therefore, the milk productivity of the indigenous cattle is very low. These large number of non descript indigenous cattle have to be improved by upgrading with suitable indigenous high milk yielding breeds such as Simmental and Kilis breed. Improvement in productive and reproductive performance is the key for increase the milk production. There is need to increase the milk production in relatively low performing dairy sates to achieve future milk demand and make dairy farming sustainable for farmers.

Animal Production from Cattle in Van Province

It is considered that the size of the establishment has no effect on cattle breeding practices (Özyürek et al., 2014). Unfortunately, in most of the enterprises studied in the region, there are no suitable environments for animal husbandry. The environmental conditions must be arranged so as to be suitable for animal husbandry. In accordance with the regulation of environmental conditions, breeders must be equipped as technical information. As a result, the profits of the breeders will increase with the livestock breeding in the region and will contribute to the country's economy. Meat and milk products from cattle in Turkey is presented in Table 4.

Table 4. Meat and milk products from cattle in Turkey (tons) (TOB, 2020).

Year	Meat	Milk
2015	1014926	16933520
2016	1059195	16786263
2017	987482	18762319
2018	1003859	20036877
2019	1201469	22960379

In Turkey, there are serious problems in the use of litter that provides dryness and softness in the sleeping and resting places of dairy cattle farms. In large-scale researches conducted in 2016, it is observed that the bodies of cows in milk processing are unacceptably dirty (up to 70%), thus causing frequent foot, breast and reproductive health and milking hygiene problems (TOB, 2020). Meat and milk products from cattle in Van province is shown in Table 5.

Table 5. Meat and milk products from cattle in Van province (tons) (TOB, 2020).

Year	Meat	Milk
2015	10047	153543.45
2016	10486	148939.61
2017	9776	187412.42
2018	9938	177268.80
2019	11894	175480.19

It is extremely important that the breeders and the organizations engaged in animal husbandry

have knowledge of occupational health and safety. The nature of livestock husbandry requires organization that is its own appropriate in accordance with local conditions for the occupational health and safety. These organizations should be units that are tried to be prevented by determining at the source of the danger. For this aim, the risks at work should firstly be determined. Then, solution suggestions should be presented to remove or minimize these risks.

Zoonotic diseases are one of the most important problems of workers in cattle husbandry. Workers and animals must be vaccinated against various zoonotic diseases. The rules of order and hygiene must be take into accounted during the milking and the shearing of the animals. Improved water supply should be combined with improved sanitation, special needs of workers, and a separate toilet in each household to facilitate personal hygiene. Taking precautions for occupational health and safety are very difficult, costly and time consuming. Also, not all agricultural activities carry the same risk, and, as noted above, there are many special populations that must be considered.

Conclusion

Van province has ecological conditions suitable for animal production. In Van province of Eastern Anatolia, cattle husbandry participates with 2.5% in general livestock production in Turkey with high share. Characteristics of cattle husbandry in Van province are small farms with 2 to 3 cows per farm (80-85% of total number of farms). The most important of native cattle breeds of Turkey is the Eastern Anatolia Red breed. The cow milk from this breed is either consumed by the household as raw milk or processed as herbaceous cheese. As a result, preservation and development of native cattle breeds as a genetic source is very important. If the current potential especially in the Eastern Anatolian Region and in Turkey is evaluated, it can become very important in this region. For cattle presence and animal production in Van province, it can be said that it has an important place in terms of its features.

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OCCUPATIONAL ACCIDENTS, DISEASES AND PREVENTIONS AT SMALL RUMINANT HUSBANDRY IN EASTERN ANATOLIA OF TURKEY

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Abstract

The aim of this review is to provide sensitivity about occupational health and safety, accidents, diseases, and musculoskeletal disorders in small ruminant husbandry. Animal production is associated with a variety of occupational illnesses and injuries. In small ruminant husbandry, the most important task in the care-feeding and management of animals falls into workers. Occupational Health and Safety (OHS) has significant economic implication particularly in terms of medical costs and economic productivity losses. The livestock activities in Turkey have been characterized by the different regional applications. The activities of small ruminants husbandry are especially an important source of income for the indigenous people in the Eastern Anatolia of Turkey. The occupational health and safety in livestock production is very important as it is in many other areas. In this paper, it has been discussed the hazards and the risks related to occupational health and safety in small ruminant husbandry. The most common hazards at small ruminant husbandry in Turkey are the ergonomics, the noise, the air conditioning, the chemicals, the occupational diseases, the zoonotic diseases, the animal attacks, the bites, the injuries, the accidents in transport, the psychological stress and, the skin-borne diseases etc. Occupational diseases and accidents that can be encountered by workers have caused the losses of very serious economic and the qualify persons in small ruminant husbandry. Therefore, the precautions related to the occupational health and safety must be taken for workers at the husbandry and the field. In Turkey, it is not possible to say that the preventive measures on the occupational health and safety in small ruminant husbandry are still sufficient. This information has been prepared based on the personal observations and the experiences directly in the local area.

Keywords: *Occupational health, Occupational safety, Sheep husbandry, Zoonotic disease.*

Introduction

The livestock activities in Turkey have been characterized by the different regional applications. Animal production has an important place in agricultural activities. There are many factors which limit the economic efficiency for production. One of them is production losses due to the workplace accidents and the occupational illness. The issue of occupational health and safety in animal production is very important as it is in many other areas. Occupational diseases and accidents that can be encountered by workers at business have caused the losses of very serious economic and the qualify person in animal production. In addition, the sustainability of production is negatively affected. The most common hazards at the animal production in Turkey are the zoonotic diseases, the ergonomics, the noise, the air conditioning, the chemicals, the animal attacks, the bites, the injuries, the accidents in transport, the psychological stress and, the skin-borne diseases etc. Especially, the animal hitting and the zoonotic diseases are very important in animal husbandry. Therefore, the precautions related to the occupational health and safety must be taken for the workers at the livestock enterprises, the field and, the factories such as the feed, the skin and the meat (Aygün et al., 2019).

The risk factors that the livestock workers faced vary according to the sector. In sheep-goat husbandry, the most important task in the care-feeding and management of animals falls into workers. Occupational Health and Safety (OHS) has significant economic implication particularly in terms of medical costs and economic productivity losses.

Sheep and goat breeding in Anatolia has been adapted to regional differences and has been characterized by the prominence of different applications (Aygün, 2017). It is true that more occupational health and safety intervention research focusing on preventing illness and injury needs to be conducted. Conducting this type of research is difficult and time-consuming; however, without increasing the number and methodological rigor of these studies, it will be difficult to identify effective intervention methods and confidently encourage their use (Goldenhar and Schulte, 1996). Animal production is associated with a variety of occupational illnesses and injuries.

The aim of this review is to provide sensitivity about occupational health and safety, musculoskeletal disorders, general problems, zoonotic diseases, and preventions needed in small ruminant husbandry. The work-related accidents encountered workers in the plateau have also been emphasized. This information has been prepared based on the personal observations and the experiences directly in the local area.

The Place and Importance of Small Ruminant Husbandry in Eastern Anatolia of Turkey

The livestock activities in Turkey have been characterized by the different regional applications. The activities of small ruminants husbandry are especially an important source of income for the indigenous people in the Eastern Anatolia of Turkey.

Sheep and goat husbandry is an industrial sector that they transform the natural vegetation cover pasture and the pasture not used in the agriculture into the products such as meat, milk and, wool. There are breeds such as White Karaman, Red Karaman, Awassi, Dağlıç, Kıvırcık, and Karayaka among local sheep breeds of Turkey. Small ruminant husbandry is indispensable and an important source of income for farmers in Van province. Van province is suitable for small ruminant breeding in terms of large pasture areas, water resources, and climate characteristics (İnan and Aygün, 2019; Aşkan ve Aygün, 2020). It can be said that the province is rich in terms of underground and surface irrigation sources as well as a suitable land structure for the production of forage crops. Small ruminant husbandry is a major industrial sector in the Eastern Anatolia of Turkey and relies heavily on migrant and nomadic farm life.

Commonly, it was aimed to be offered some information about living culture and stockbreeding activities of nomadic tribes that they come from the south to east because of weather warming up with. Studies made by some researchers in order to achieve their political goals on political and ideological studies relating to a multi-ethnic structure and stockbreeding activities that is the main source of income of the politicized tribes were the main theme of this study. One of the most important issues to be taken into account for sustainable small ruminant breeding in the region is no doubt nomadic living culture of tribes that they presented this culture and breeding system to nowadays. Some researchers have emphasized that culture of communities consisted of some narrow range cultures, and that national culture also consisted of many local, regional, or sub-cultural backgrounds (Aygün et al., 2013).

The Most Common Occupational Accidents in Small Ruminant Husbandry

Animal production is associated with a variety of occupational illnesses and injuries. In small ruminant husbandry, the most important task in the care-feeding and management of animals

falls into workers. Occupational Health and Safety (OHS) has significant economic implication particularly in terms of medical costs and economic productivity losses. The hazard is anything that has the potential to harm. Hazard can affect the person, the material and the process. Also, hazards can cause accidents, diseases, loss of product, and machine damage etc. The occupational risk refers to the combination of the likelihood and severity of an injury or illness resulting from exposure to a hazard.

Workers who are away from social habitats and who work in the hills may be exposed to allergies or poisoning caused by the attack of various wild animals, such as bee or insect bites, as well as plants grown in the spring, pollen of fungi or various flowers. Employees are camels exposed to the sun because the work area is mostly open space. Therefore, excessive exposure to sunlight can cause dermatological problems.

Since livestock workers spend a great deal of time outdoors, they are at risk for physical stress from excessively cold and excessively hot environments. The magnitude of heat and cold stress problems in agriculture is not well documented. Tolerance to such environments varies among individuals and may be difficult to predict. Livestock workers should be provided the means to compensate for extremes of temperature. For example, adequate water supplies while working outdoors in hot climates are essential.

Data on work injuries are not as readily available for berivans and shepherds in the nomadic small ruminant husbandry as for workers in other industries. Because it is difficult to keep such statistics. The number of farmers in the Turkey affected by pesticides is unknown. Little is known about the extent or magnitude of chronic health problems related to occupational exposure to pesticides. Although difficult, it is important to carry out further studies on the adverse health effects associated with pesticides among farm workers. Migrant farm workers have exposure to other hazards that may increase their risk of health problems: climate-dependent problems, such as heat stroke or cold shock, and occupationally caused infections such as anthrax, ascariasis, encephalitis, leptospirosis, rabies, salmonellosis, tetanus, and coccidioidomycosis. Sensory problems are common: eye problems, caused by irritation, infection, or injury from the wind, sun, dust or soil, agricultural chemicals, debris ejected from farm machinery, and allergic reactions to plants, and hearing problems due to noise from farm machinery and cannery work.

The Most Common Occupational Diseases in Small Ruminant Husbandry

An "occupational disease" is any disease or disorder contracted primarily as a result of an exposure to risk factors arising from work activity. "Work-related diseases" have multiple causes, where factors in the work environment may play a role, together with other risk factors, in the development of such diseases. The World Health Organization emphasizes the following: Carrying our estimates of the global burden of disease from major occupational risks, such as injuries, airborne exposures, carcinogens, ergonomic stressors, noise and other specific risks. Incorporating occupational diseases and their causes in the 11th revision of the International Statistical Classification of Diseases and Related Health Problems. Working with ILO to develop diagnostic and exposure criteria for occupational diseases and to enable primary and secondary health care providers to detect and report such diseases (WHO, 2020).

The most common hazards at small ruminant husbandry in Turkey are the ergonomics, the noise, the air conditioning, the chemicals, the occupational diseases, the zoonotic diseases, the animal attacks, the bites, the injuries, the accidents in transport, the psychological stress and, the skin-borne diseases etc. Occupational diseases and accidents that can be encountered by workers have caused the losses of very serious economic and the qualify persons in small ruminant husbandry. Therefore, the precautions related to the occupational health and safety must be taken for workers at the husbandry and the field (Aygün, 2017).

Zoonoses is naturally called vertebrate animals to humans, and humans to animals to diseases or infections. Zoonoses are infections that are spread from animals to humans. The World Health Organization (WHO) defines zoonotic diseases as 'any diseases or infections that are naturally transmitted between vertebrate animals and humans.' Agents causing zoonotic diseases may be bacteria, fungi, viruses, parasites or any other communicable agents, for example prions. Currently there are over 200 recognised zoonoses, some of which have a worldwide distribution and others which are localised to specific regions. The situation is not static, and emerging zoonotic diseases are continually being recognised, both animal diseases which have spread to humans for the first time and existing zoonoses spreading to new geographical areas. Occupational zoonotic diseases are most common where there is close contact between animals and humans at work, for example in animal husbandry and agricultural occupations, although workers in a wide range of other occupations may also be exposed to zoonotic agents, including those employed in the outdoor leisure industry or the waste water industry and laboratory workers. There are many occupational zoonotic diseases in the world, many of which occur very rarely, although some do pose a significant health risk for workers in certain occupations. While the incidence of specific zoonoses varies from country to country, there are many occupational zoonoses that occur across Europe, although not every disease is present in every country. (Cook and Farrant, 2020)

Other health and safety risks include skin problems, hearing loss, stress, and mental well-being issues particular to farming and the rural way of life. Occupational skin disorders are common in livestock workers. The effects of sun exposure are an important cause of morbidity in berivans and shepherds group.

Berivans and shepherds' lung is one of many forms of *hypersensitivity pneumonitis*. This problem is becoming rare, which is likely due to the reduction of exposure to organic dust from the increasing mechanization of agriculture and the effect of livestock health and safety programs (Von Essen and McCurdy, 1998).

Another danger for berivans and shepherds is the waste of animals. Animal wastes are frequently stored underground and are a source of toxic gases. Entering confined spaces used for manure storage can lead to fatalities, which are often caused by hydrogen sulfide exposures (Von Essen and McCurdy, 1998).

Some Suggestions and Possible Preventions

It is extremely important that the breeders and the organizations engaged in animal husbandry have knowledge of occupational health and safety. The nature of nomadic small ruminant husbandry requires organization that is its own appropriate in accordance with local conditions for the occupational health and safety. These organizations should be units that are tried to be prevented by determining at the source of the danger. For this aim, the risks at work should firstly be determined. Then, solution suggestions should be presented to remove or minimize these risks.

Zoonotic diseases are one of the most important problems of berivans and shepherds in nomadic animal husbandry. Workers (berivans and shepherds) and animals must be vaccinated against various zoonotic diseases.

With regard to the control of occupational zoonoses, there are some general control measures which reduce the risk of infection for a wide range of zoonoses. These include the following:

- good personal hygiene practices, especially washing with soap and warm water;
- covering cuts and scratches with waterproof dressings;
- wearing of appropriate PPE, for example gloves, overalls, respiratory protection – this must provide relevant protection, while also being suitable for carrying out the required task;

- good hygiene practices for animal husbandry; and
- use of an appropriate disinfectant to clean potentially contaminated areas.

For certain zoonoses there is an effective vaccine available and it may be appropriate to administer this to individuals in high risk occupations, for example laboratory workers handling infected animals. In many cases there are effective prophylaxis and treatments available. For these to be used to maximum advantage, it is necessary for workers to be aware of any diseases they may be at risk from and to be able to recognise early symptoms of these diseases. For certain occupations it may be required for workers to inform their employer if they have a weakened immune system (Cook and Farrant, 2020).

The rules of order and hygiene must be take into accounted during the milking and the shearing of the animals. Improved water supply should be combined with improved sanitation, special needs of women workers, and a separate toilet in each household to facilitate personal hygiene.

These approaches are necessary to obtain the cooperation of nomadic workers and their employers so that occupational exposures and protection as well as health consequences are accurately and completely ascertained. In addition, information about health effects should be obtained in a way that is not only culturally sensitive but also meaningful to study participants and yet comparable to that obtained through standardized instruments. Undertaking studies of occupational health risks in this population with these considerations will not only contribute to the understanding of such risks but can also further preventive efforts and lead to better health in this high-risk population. Effective prevention can reduce suffering and death and contribute to enhanced productivity in the workplace. In this way, both the employers and the employees gain (Aygün and Demir, 2015).

Taking precautions for occupational health and safety are very difficult, costly and time consuming. Among the difficulties is the varied nature of agriculture, the many ethnic groups engaged in the activities, the traditionalist view of farming families, and rapidly changing technology. Also, not all agricultural activities carry the same risk, and, as noted above, there are many special populations that must be considered.

Conclusion

The work-related accidents encountered workers in the plateau have also been emphasized. This information has been prepared based on the personal observations and the experiences directly in the local area. There are many factors which limit the economic efficiency for production. One of them is production losses due to the workplace accidents and the occupational illness. The issue of occupational health and safety in animal production is very important as it is in many other areas. In addition, the sustainability of production is negatively affected. Occupational diseases and accidents that can be encountered by workers have caused the losses of very serious economic and the qualify persons in small ruminant husbandry. Therefore, the precautions related to the occupational health and safety must be taken for workers at the husbandry and the field. There are a number of characteristics of Turkish agriculture that need to be acknowledged for an effective occupational health and safety response to the farm injury or illness problem. In Turkey, preventive measures have started to be taken on occupational health and safety in livestock production.

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TRANSHUMANCE ACTIVITIES IN SMALL RUMINANT HUSBANDRY IN VAN PROVINCE OF EASTERN ANATOLIA IN TURKEY

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Abstract

In this paper, the transhumance activities and the production habits of breeders in sheep and goat husbandry have been discussed. This information has been prepared based on the personal observations and the experiences directly in local area. The livestock activities in Turkey have been characterized by the different regional applications. The activities of small ruminants husbandry are especially an important source of income for the indigenous people in Van province of Eastern Anatolia in Turkey. In the highland sheep husbandry, sheep herds are taken to towards the end of the spring the plateaus which are cool and grassy with the drying of pastures and the start of heat. For a period of 3-5 months, sheep remain in control by shepherds in highland. After the weather cools down, highland breeders and sheep return to villages or to their settlements again plain. Sheep herds usually consist of 300 to 500 heads. Sheep herds were formed by gathering business owners with a different number of animals. Sheep herds are taken to the highlands by grazing or by road transport. One of the most important examples of livestock farming is highland small ruminant husbandry in Van province. The pastoralism refers to season stay at the high plateaus for small ruminant production and semi nomadic life. In order to get more abundant products such as milk, cheese, wool and so on, the people of the region have to go to highlands with the arrival of spring animals to find better grazing and water areas. In this province, it is recommended to keep records in order to obtain a sustainable income source from small ruminant husbandry.

Keywords: *Breeding model, Goat breeding, Sheep breeding, Transhumance activities.*

Introduction

Eastern Anatolia Region of Turkey has a different character than other regions in terms of climate conditions. In the winter months, when there are severe storms in this region, extreme cold occurs. Van is one of the most prominent cities of this area in which its public sustain themselves by animal production. The socio-economic and cultural structure, climate and topographic features of the region have played a role in gaining importance of sheep breeding. Sheep breeding has a special place and importance in animal husbandry in Van province. According to the investigations in the region, pasture-based animal husbandry and, accordingly, sheep breeding are dominant (İnan and Aygün, 2019). For this reason, it is very important to define the condition, potential, and problems of breeding sector especially with respect to the small ruminant husbandry in this city. It can be said that the fact that sheep breeding is carried out in herd rather than management level at the enterprise level has an important effect on the spread of plateau and nomadic sheep farming with the effect of structural difference. This situation is more evident in the Eastern and Southeastern Anatolia Region in Turkey. In this sense, since sheep farming is mostly done by nomadic families or tribes, it is important to compile information about this section and to determine the general characteristics of the nomadic families (Aşkan and Aygün, 2020). Animal husbandry is one of the most important economic sectors of Turkey. Sheep production systems in Turkey depend on factors such as the natural and socio-economic conditions of the regions, the availability of

feed resources, the connection to plant production and, the consumption habits of the population. These are systems of the stock breeding, the highland sheep husbandry, and the nomadic livestock breeding (Kaymakçı, 2010). Livestock in Eastern Anatolia province are mostly carried out with traditional methods. Therefore, it will provide contribution to knowledge of the current problems of the industry in the province the current situation of the livestock sector and needs to be determined and the current situation to the steps taken to improve (Şeker and Köseman, 2015). In this paper, the transhumance activities and the production habits of breeders in sheep and goat husbandry have been discussed. This information has been prepared based on the personal observations and the experiences directly in local area.

Demographic Structure of Van Province

Van province is located in Lake Van in Eastern Anatolia Region Chapter of the Upper Murat-Van in a closed basin on Turkey. The border with the Iranian State lies to the east of Van. Van is surrounded by Van Lake from west. It is also surrounded by cities such as Agri from north and Bitlis from west, Siirt, Şırnak, Hakkari from south and Iran from east. Van city is nineteenth the most crowded city in Turkey. As of the end of 2019, its population is 1.136.757 people. Van is the fifth largest province in terms of surface area in Turkey. Its area is 21.334 km^2. Although Van Lake and its valleys are covered with rich vegetation, the mountains are generally barren (Anonymous, 2020). Economy of Van province is an economy mainly based on agriculture and animal husbandry, aquaculture, and tourism. Small ruminant husbandry, cattle breeding, and especially the last poultry farming in recent years. Livestock sector in Van province every is developing day by day and gaining importance.
As Van province is in the lower status in terms of socio-economic development (in the 5th level), its unemployment rate high and employment rate low, and more than half of the working population in different sectors is concentrated on the agriculture sector significant migrations occurred in the province (Elmastaş and Yılmaz, 2015). The educational status of the cattle farmers and families of Van province is low. This will have a negative impact on the care and feeding of animals and the productivity of the products to be obtained. For this purpose, meetings where business owners can get technical information can be organized and small units can be created and information can be transferred from village to village. Thus, it will be contributed both to the conscious raising of animal husbandry and the economy of the country.

The Importance of Small Ruminant Husbandry in Van Province of Eastern Anatolia Region in Turkey

Animal husbandry is one of the most important economic sectors of Turkey. Sheep production systems in Turkey depend on factors such as the natural and socio-economic conditions of the regions, the availability of feed resources, the connection to plant production and, the consumption habits of the population. These are systems of the stock breeding, the highland sheep husbandry, and the nomadic livestock breeding.
The livestock activities in Turkey have been characterized by the different regional applications. The activities of small ruminants husbandry are especially an important source of income for the indigenous people in Van province of Eastern Anatolia in Turkey.
Sheep husbandry is an industrial sector that they transforms the natural vegetation cover pasture and the pasture not used in the agriculture into the products such as meat, milk and, wool. There are breeds such as White Karaman, Red Karaman, Awassi, Dağlıç, Kıvırcık, and Karayaka among local sheep breeds of Turkey (İnan and Aygün, 2019). Small ruminant

husbandry is indispensable and an important source of income for farmers in Van province. Van province is suitable for small ruminant breeding in terms of large pasture areas, water resources, and climate characteristics. It can be said that the province is rich in terms of underground and surface irrigation sources as well as a suitable land structure for the production of forage crops.

In the highland sheep husbandry, sheep flocks are removed to the highlands with cool and plenty of grassy plains by pressing hot and dry towards the end of spring. For a period of 3-5 months, sheep remain in control by shepherds in the highland. After the weather cools down, sheep go back to the villages or the farms in the plain. Sheep herds usually consist of 300 to 500 heads. Each sheep is composed of lots of different people with a lot of expenses, depending on the number of animals contributes. Sheep herds were formed by gathering business owners with a different number of animals. Sheep herds are taken to the highlands by grazing or by road transport.

Transhumance Activities in Small Ruminant Husbandry in Van Province of Eastern Anatolia Region in Turkey

One of the most important examples of livestock farming is highland small ruminant husbandry in Van province. The pastoralism in Van is one of the most beautiful life style. The pastoralism is refer to the exit the high plateaus for small ruminant production and semi nomadic life. In order to get more abundant the products such as milk, cheese, wool and so on, the people of the region have to go to the highlands with the arrival of spring animals to find better grazing and water areas. With the arrival of spring to the first zone in the region is exited. With the start of the cold days of autumn again return to the settlements. In this province, it is recommended to keep records in order to obtain a sustainable income source from small ruminant husbandry. Small ruminant husbandry is an industrial sector that they transforms the natural vegetation cover pasture and the pasture not used in the agriculture into the products such as meat, milk, and fleece. Small ruminant breeding is indispensable and an important source of income for farmers in Van province. Van province is suitable for both small ruminant breeding and cattle husbandry in terms of large pasture areas, water resources, and climate characteristics. The purpose of sheltering animals is to eliminate the negative effects of the environment on animals within economic limits and to provide comfortable living conditions suitable for their behavior. For this reason, when designing animal shelters, they should be dimensioned so as to provide sufficient space and internal detail for the movement, social, feed and water drinking behaviors of animals, and should be kept within economic and optimal limits in care management and hygienic conditions (Mutaf et al., 2001). It can be said that the province is rich in terms of underground and surface irrigation sources as well as a suitable land structure for the production of forage crops. However, small ruminant husbandry is a major industrial sector in Van province in the Eastern Anatolia of Turkey (İnan and Aygün, 2019). Meanwhile, it is directly dependent on the involvement of the breeder to be successful in all studies to improve breeding and environmental factors at the breeder level. The numbers of indigenous sheep and goat are high and there is need for conservation and spread of indigenous pure breeds on other suitable areas. In Van province of Eastern Anatolia in Turkey, the indigenous sheep and goat were found more in numbers. But the milk productivity of the indigenous sheep and goat breeds is very low. Therefore, improvement in productive and reproductive performance of sheep and goat is the key for increase the milk production. There is need to increase the milk production in relatively low performing dairy sates to achieve future milk demand and make dairy farming sustainable for farmers.

The populations of sheep and goat in its native tract is decreasing steadily and there is no information on its present status in its home tract. Pure animals are still found in and around Van province. The sheep-goat breeders of this region are forced to migrate from the home tract to the cities along without their flocks and herds, due to economical and social problems of the region. Migration realised during the 20 years. In this process, they sold their animals. During the course of migration, the number of sheep and goat in the region decreased steadily. In the current time they are returning back to their dwellings systematically.

Overview of the General Problems of Small Ruminant Breeders in Highland

One of the most important issues to be taken into account for sustainable small ruminant breeding in the region is no doubt nomadic living culture of tribes that they presented this culture and breeding system to nowadays. Some researchers have emphasized that culture of communities consisted of some narrow range cultures, and that national culture also consisted of many local, regional, or sub-cultural backgrounds. Unfortunately, in most of the farms studied in the region, there are no suitable environments for small ruminant husbandry. The environmental conditions must be arranged so as to be suitable for animal husbandry. In accordance with the regulation of environmental conditions, breeders must be equipped as technical information. As a result, the profits of the breeders will increase with the livestock breeding in the region and will contribute to the country's economy. In this region, there are serious problems in the use of litter that provides dryness and softness in the sleeping and resting places of small ruminant farms. In large-scale researches conducted in last years it is observed that the bodies of animal in milking and shearing processing are unacceptably dirty, thus causing frequent foot, breast and reproductive health, shearing, and milking hygiene problems (TOB, 2020). It is extremely important that the breeders and the organizations engaged in animal husbandry have knowledge of occupational health and safety. The nature of livestock husbandry requires organization that is its own appropriate in accordance with local conditions for the occupational health and safety. These organizations should be units that are tried to be prevented by determining at the source of the danger. For this aim, the risks at work should firstly be determined. Then, solution suggestions should be presented to remove or minimize these risks. Zoonotic diseases are one of the most important problems of workers in cattle husbandry. Workers and animals must be vaccinated against various zoonotic diseases. The rules of order and hygiene must be take into accounted during the milking and the shearing of the animals. Improved water supply should be combined with improved sanitation, special needs of workers, and a separate toilet in each household to facilitate personal hygiene. Taking precautions for occupational health and safety are very difficult, costly and time consuming. Also, not all agricultural activities carry the same risk, and, as noted above, there are many special populations that must be considered.

Sevinç (1972) reported in her study in this region that collected the solutions of the problems of nomadic ovine livestock in 5 items in general and said that it is necessary;

-To protect the pasture and meadow vegetation in the winter lands with the highland and highland slopes and to take continuous measures to ensure their development,

-To identify and secure migration routes in the most convenient way,

-To provide a continuous and secure market order for migratory sheep breeders,

-To improve and increase the efficiency of the nomadic ovine animals with effective measures compatible with the lives of migratory ovine livestock families,

-To bring order to a structure that will modernize ovine livestock within a certain period of time for migratory sheep breeders. Unfortunately, it can be said that these problems are still not resolved today.

One of the most important problems of the immigrants in the region is undoubtedly related to the education of our children, especially girls, which is emphasized in every platform that is important in the future of the country. The children who contribute to their families by staying in tents on the highland, take part in fulfilling the requirements of life in all conditions of nomadic life. They can always start their education life one step behind. Girls do not even have such a chance, in this way of life.

Conclusion

In this paper, the transhumance activities and the production habits of breeders in sheep and goat husbandry have been discussed. This information has been prepared based on the personal observations and the experiences directly in local area. Van province has ecological conditions suitable for animal production. If the current potential especially in Van province is evaluated, it can become very important in Eastern Anatolia Region of Turkey. For sheep and goat presence and animal production in Van province, it can be said that it has an important place. It is hoped that it has now been understood by the state that migratory breeders, which have been neglected in many ways for many years, are people engaged in animal production in the Eastern Anatolia Region of Turkey.

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THE INFLUENCE OF THE AGE OF LAYING HENS ON THE DAILY FEED CONSUMPTION PER LAYING HEN AND EGG

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Abstract

Feed consumption is one of the most essential parameters of the poultry production, because the costs of nutrition often make over 60% of the total cost. Today's light line hybrids, selected for the production of eggs for consumption, realize exceptional conversion of food to egg mass. From an economic point of view, the information about feed consumption per product unit, i.e. produced egg, is more interesting in relation to the daily consumption of food per laying hen, which is not the best indicator of economic production. Since it has been established in the area of the Balkans, that commercial flocks in the production of the eggs are held to the end of the 72nd week of age as a final instance in the production, the aim of this paper is to examine what is going on with feed consumption per hen, and produced egg, before, as well as, after established 72nd week of age, up until the end of the 79th week of age of hen hybrids Lohmann Brown, where the production period lasted 61 weeks, which is 427 days. The examinations were performed on the light line hybrid Lohmann Brown by conducting appropriate experiment on the laying hens' farm "Agrovet" in Foča municipality, Bosnia and Herzegovina – Entity of Republic of Srpska. During the research, the following basic indicators were tested: food consumption (daily per laying hen and per produced egg) by weeks and for the entire production cycle. We also determined the coefficients of phenotype correlation between the age and daily feed consumption per laying hen, or per produced egg. By calculating the phenotypic correlation between the age of laying hens, and the daily feed consumption per laying hen and produced egg, it can be concluded that, in the present case, it is justified to use laying hen in the production of eggs for 61 weeks. Viewed as a whole, it can be concluded that the analyzed commercial flock of light line hybrids Lohmann Brown, in most tracked production indicators has achieved satisfactory results which values are mostly within the limits of the values to the technological standards predicted by selector.

Keywords: *eggs for consumption, feed consumption per laying hen, feed consumption per produced egg, correlation.*

Introduction

Food consumption is one of the most essential parameters of the poultry production, because the costs of nutrition often make over 60% of the total cost. Today's light line hybrids, selected for the production of eggs for consumption, realize exceptional conversion of food to egg mass, which is on the verge of reaching its biological maximum (Rakonjac, 2017). Pandurević et al. (2016) think that, from an economic point of view, the most interesting information is about feed consumption per unit of product, i.e. per produced egg. Also, feed consumption per one egg depends on the mass of the egg, the bigger the egg, the bigger is feed consumption, in addition to all other factors equal, and vice versa. On the basis of previous observations, the daily consumption of feed per laying hen is not the best indicator

of economic production, and the advantage is given to feed consumption per produced egg. Success in the production of eggs depends on the degree of providing optimal conditions: securing sufficient quantities of high quality feed and water; securing appropriate climate in facilities (temperature, air composition, humidity, illumination); usage the proper equipment, which reduces the possibility of harming the hens and breaking the eggs. Babić (2015) in his work concludes that hens in enriched cages consumed more feed, the feed consumption per egg was bigger, and feed conversion was worse, compared to hens kept in conventional cages. Feed consumption per egg moved around 140 g with laying hens kept in conventional cages, while it amounted to around 150 g in enriched cages. Due to the possibility of moving in cages, the hen spent more energy and feed conversion was worse, i.e. the consumption of feed for a kilo of eggs mass was worse. Pandurević et al. (2016) conclude that the average daily consumption of feed during the period of egg production (20-72 week of age) amounted to 126.97 g per laying hen, and 151.47 g per produced egg. The maximum daily consumption of feed per laying hen amounted to 139.29 g (50th week of age), and the minimum 112.32 g (20th week of age), while for a specified period of time each laying hens consumed an average of 47 kg of feed. Daily consumption of feed per laying hen gradually increased until the middle of the production cycle, then stagnated, and towards the end, decreased to a certain extent. By the 45th week of age, the strong positive correlation was found between the daily feed consumption and the age of the hen, by the 58th week of age the correlation was very strong, by the 65th week of age correlation was strong, and by the 70th week of age the correlation was weak. Unlike the daily feed consumption per laying hen, the correlation coefficients between the age of laying hens and the consumption of feed per produced egg were negative and statistically significant ($P<0.05$) up to 48th week of age, and after this period there was no statistical significance ($P>0.05$). The analysis of movement of body mass, growth and feed consumption of observed egg-laying hens in the course of the experiment Petričević (2014) concludes that the obtained production results are within the limits of the technological standards for observed provenience Isa Brown (the brown color the shell). Statistical analysis of the data also determined that the examined factors did not show the influence, either individually or collectively, to the consumption of feed. Feed consumption during entire period of the research ranged in the interval from 114.4 g to 116.1 g. Examining the production of eggs for consumption, with hybrids for the production of eggs with brown shell Isa Brown, Pandurević et al. (2016), determined that the daily feed consumption per laying hen amounted to 120.28 g on average, while the average consumption of feed per produced egg was 145.49 g. The obtained results are in accordance with the technological standards of selector of hybrids Isa Brown.

Daily food consumption of 120 g per laying hen as well as 144.5 g of feed per egg, were obtained with hybrids for the production of eggs with brown shell Hisex Brown, Gjorgovska et al. (2016). Since it was established in the area of the Balkans, that commercial flocks in the production of the eggs are held to the end of the 72nd week of age, as a final instance in the production, the aim of this paper is to examine what is going on with feed consumption per hen before and after established 72nd week of age. In this paper we talk about a period of seven weeks longer, up until the end of the 79th week of age of hen hybrids Lohmann Brown, where the production period lasted 61 weeks, which is 427 days.

Material and Methods

As the initial material for the research, we used 7.720 hens of light line hybrids Lohmann Brown, placed into the facility on the farm "Agrovet" Foča, with at least 18 weeks of age, i.e. the experiment started in the 19th week of age of laying hens. The production period lasted for 61 weeks (the age of the laying hens 79 weeks). A farm for the production of eggs for

consumption on which the experiment was performed uses a classic battery (cage) system to accommodate hens. In the course of raising (exploitation) of the commercial flocks, a technology proposed by the selector of laying hens hybrid was used (http://www.ltz.de). All technological phases (feeding, power, temperature, lighting, ventilation, drainage system and collecting the eggs) are automatically regulated. According to the recommendations of the selector (http://www.ltz.de), the breeder of a line of light hybrids Lohmann Brown, the forage during the production cycle should contain from 2,750 to 2.800 kcal ME and 17.5% of crude protein (load capacity below 5%), or 2,800 kcal ME and about 18% of crude protein (capacity over 5%). During the exploitation of commercial flocks of laying hens, the special attention was paid to four production phases: 27th week of age - WA27 ("a peak" – the maximum produced eggs), 46th week of age - WA46 (mid production cycle), 72nd week of age - WA72 (the usual end of the production cycle) and 79th week of age of laying hens (the end of the production cycle of laying hens in the experiment). In the course of the duration of experiment, the following indicators are examined: feed consumption (per laying hen and per produced egg) by the weeks and for the entire production cycle (61 weeks) from 19th to 79th week of age of laying hens (WA19 - WA79). In order to determine the consumption of feed, the records are kept on daily basis. Based on the data, i.e. by dividing the weekly food consumption with a number of laying hens, then obtained quotient with the number seven, and then multiplying with 1,000, the average daily food consumption per laying hen (g) was calculated. Weekly feed consumption per laying hen (g) was calculated by dividing weekly food consumption with the number of laying hens, and multiplying the obtained result with 1.000. Based on the weekly food consumption and the number of produced eggs, the feed consumption (g) for the production of one egg is calculated. On the basis of the obtained data, the appropriate database was established. The software package SPSS-Statistical Package for Social Sciences (http://spss.en.softonic.com/) was used for statistical analysis. Based on the obtained results, the phenotypic correlation between the age of commercial flocks of laying hens and the daily feed consumption per laying hen and per produced egg, have been established and tested. The power of the phenotype correlation was discussed on the basis of the Roemer-Orphal`s classification (Latinović, 1996).

Results and Discussion

The total amount of consumed feed per weeks of the production during the entire production cycle, then daily and weekly feed consumption per laying hen, as well as the feed consumption per produced egg is presented in the table 1.

Table 1. Feed consumption per weeks of production during the production cycle.

Weeks of age/production	Total amount of feed (kg)	Weekly per laying hen (g)	Daily per laying hen (g)	Weekly eggs per laying hen	Feed per laying hen (g)
$WA_{19/1}$	5,460	707.25	101.04	2.01	351.87
$WA_{20/2}$	5,600	725.39	103.63	4.36	166.37
$WA_{21/3}$	5,600	725.39	103.63	4.36	166.37
$WA_{22/4}$	5,670	743.46	104.92	6.27	117.14
$WA_{23/5}$	5,740	743.52	106.22	6.58	113.00
$WA_{24/6}$	5,780	748.70	106.96	6.63	112.93
$WA_{25/7}$	5,800	751.30	107.33	6.63	113.32
$WA_{26/8}$	5,840	756.48	108.07	6.57	115.14
$WA_{27/9}$	5,880	761.66	108.81	6.63	114.88
$WA_{28/10}$	5,920	766.84	109.55	6.55	117.08

$WA_{29/11}$	5,950	770.73	110.10	6.52	118.21
$WA_{30/12}$	6,000	777.20	111.03	6.53	119.02
$WA_{31/13}$	6,050	783.68	111.95	6.52	120.20
$WA_{32/14}$	6,090	788.86	112.69	6.52	120.99
$WA_{33/15}$	6,120	792.75	113.25	6.50	121.96
$WA_{34/16}$	6,140	795.34	113.62	6.45	123.31
$WA_{35/17}$	6,160	797.93	113.99	6.42	124.29
$WA_{36/18}$	6,160	797.93	113.99	6.36	125.46
$WA_{37/19}$	6,200	803.11	114.73	6.33	126.87
$WA_{38/20}$	6,200	803.11	114.73	6.33	126.87
$WA_{39/21}$	6,230	806.99	115.28	6.32	127.69
$WA_{40/22}$	6,230	806.99	115.28	6.28	128.50
$WA_{41/23}$	6,260	810.88	115.84	6.24	129.95
$WA_{42/24}$	6,300	816.06	116.58	6.25	130.57
$WA_{43/25}$	6,300	816.06	116.58	6.17	132.26
$WA_{44/26}$	6,265	811.53	115.93	6.00	135.26
$WA_{45/27}$	6,230	806.99	115.28	5.98	134.95
$WA_{46/28}$	6,160	797.93	113.99	6.18	129.12
$WA_{47/29}$	6,160	797.93	113.99	6.17	129.32
$WA_{48/30}$	6,120	792.75	113.25	6.16	128.69
$WA_{49/31}$	6,090	788.86	112.69	6.13	128.69
$WA_{50/32}$	6,090	788.86	112.69	6.07	129.96
$WA_{51/33}$	6,055	784.33	112.05	6.07	129.21
$WA_{52/34}$	6,055	784.33	112.05	6.03	130.07
$WA_{53/35}$	6,020	779.79	111.40	6.08	128.25
$WA_{54/36}$	6,020	779.79	111.40	6.02	129.53
$WA_{55/37}$	6,020	779.79	111.40	6.02	129.53
$WA_{56/38}$	5,985	775.26	110.75	5.98	129.64
$WA_{57/39}$	5,950	770.73	110.10	5.97	129.10
$WA_{58/40}$	5,950	770.73	110.10	5.94	129.75
$WA_{59/41}$	5,940	769.43	109.92	5.85	131.53
$WA_{60/42}$	5,950	770.73	110.10	5.81	132.66
$WA_{61/43}$	5,960	772.02	110.29	5.83	132.42
$WA_{62/44}$	5,950	770.73	110.10	5.78	133.34
$WA_{63/45}$	5,915	766.19	109.46	5.70	134.42
$WA_{64/46}$	5,930	768.13	109.73	5.68	135.24
$WA_{65/47}$	5,900	764.25	109.18	5.67	134.79
$WA_{66/48}$	5,870	760.36	108.62	5.66	134.33
$WA_{67/49}$	5,840	756.48	108.07	5.60	135.09
$WA_{68/50}$	5,840	756.48	108.07	5.49	137.79
$WA_{69/51}$	5,820	753.89	107.70	5.50	136.82
$WA_{70/52}$	5,820	753.89	107.70	5.45	138.33
$WA_{71/53}$	5,800	751.30	107.33	5.39	139.39
$WA_{72/54}$	5,780	748.70	106.96	5.30	141.00
$WA_{73/55}$	5,720	740.93	105.85	5.22	141.94
$WA_{74/56}$	5,700	738.34	105.48	5.26	140.37
$WA_{75/57}$	5,680	735.75	105.11	5.18	142.04
$WA_{76/58}$	5,660	733.16	104.74	5.03	145.76

$WA_{77/59}$	5,640	730.57	104.37	4.93	148.19
$WA_{78/60}$	5,640	730.57	104.37	4.90	149.10
$WA_{79/61}$	5,620	727.98	104.00	4.95	147.07
Average	362,825	770.46	110.07	5.88	134.18

When it comes to the total consumption of feed per laying hen, we can see a constant increase in the feed consumption until WA43/25, after which comes to the phased reduction of feed consumption until the end of the production (WA79/61), with three oscillations in the 60th, 61st and 64th week of age of laying hen, when we noticed a slight increase in feed consumption compared to previous week. Table 1 shows that the feed consumption respectively increased and decreased on daily and weekly basis, in a way that feed consumption gradually increased until 43rd week of age, with oscillations in the 60th, 61st and 64th week of age, which were characteristic for the total feed consumption. Then the feed consumption on a daily and weekly level continued to decline until the end of the production cycle (WA79/61). During the experiment, an average 5.88 eggs were produced per laying hen a week, while it was spent on average 134.18 grams of feed for producing one egg. Average daily feed consumption per laying hen was 110.07 g, while it was spent an average of 770.46 g of feed per hen a week. By the end of 72nd week of age of laying hens (the established period up to when the commercial flocks in the production of eggs are held) it was produced an average of 5.99 eggs per laying hen a week, while the average were spent of feed was 132.79 g for one egg. It was spent on average 775.20 g of feed per laying hen a week, while average daily feed consumption per laying hen stood at 110.74 g, which is below the values declared as technology standard of examined hybrid (112.13 g/laying hen). Higher values for average daily feed consumption with hybrids for the production of eggs with brown shell (20-72 week of age) are noted by Milošević (2014) 120 g, Petričević (2014) 115 g, Pandurević et al. (2016), Gjorgovska et al. (2016) 120 g, and with the same hybrid, Pandurević et al. (2016) noted 126.97 g of feed per laying hen.

Higher values for the average feed consumption per produced egg with the same hybrids for the entire period of exploitation of laying hens until 72nd week of age, we can observe with Pandurevic et al. (2016), 151.47 g. Also, the higher values for the average feed consumption per egg with hybrids for the production of brown shell eggs, was found by Babić (2015) 140 g, Pandurević et al. (2016) 145.49 g and Gjorgovska et al. (2016) at 144.5 g of feed per produced egg.

On the basis of the data from table 2, we can see that there is a correlation connection and statistical significance between the age of laying hens, daily food consumption and feed consumption per produced egg.

Table 2. Phenotypic correlation between the age of laying hens, daily food consumption per laying hen (g) and food consumption per egg (g).

Weeks of age/production	Daily per laying hen (g)		Food per egg (g)	
	r_{xy}	Connection	r_{xy}	Connection
32/14	0.990***	Complete	-0.513**	Strong
33/15	0.991***	Complete	-0.490**	Medium
34/16	0.992***	Complete	-0.467**	Medium
35/17	0.991***	Complete	-0.446*	Medium
36/18	0.989***	Complete	-0.425*	Medium
37/19	0.988***	Complete	-0.404*	Medium
38/20	0.985***	Complete	-0.385*	Weak
39/21	0.984***	Complete	-0.367*	Weak

40/22	0.981***	Complete	-0.350*	Weak
41/23	0.980***	Complete	-0.332*	Weak
42/24	0.980***	Complete	-0.315^{ns}	Weak
43/25	0.978***	Complete	-0.297^{ns}	Weak
44/26	0.973***	Complete	-0.277^{ns}	Weak
45/27	0.964***	Complete	-0.259^{ns}	Weak
46/28	0.944***	Complete	-0.251^{ns}	Weak
47/29	0.926***	Complete	-0.243^{ns}	Very weak
48/30	0.902***	Complete	-0.236^{ns}	Very weak
49/31	0.874***	Very strong	-0.230^{ns}	Very weak
50/32	0.848***	Very strong	-0.222^{ns}	Very weak
51/33	0.816***	Very strong	-0.217^{ns}	Very weak
52/34	0.787***	Very strong	-0.210^{ns}	Very weak
53/35	0.752***	Very strong	-0.206^{ns}	Very weak
54/36	0.719***	Strong	-0.201^{ns}	Very weak
55/37	0.689***	Strong	-0.196^{ns}	Very weak
56/38	0.653***	Strong	-0.191^{ns}	Very weak
57/39	0.611***	Strong	-0.187^{ns}	Very weak
58/40	0.572***	Strong	-0.182^{ns}	Very weak
59/41	0.534***	Strong	-0.176^{ns}	Very weak
60/42	0.500***	Strong	-0.169^{ns}	Very weak
61/43	0.471***	Medium	-0.162^{ns}	Very weak
62/44	0.441***	Medium	-0.155^{ns}	Very weak
63/45	0.406**	Medium	-0.147^{ns}	Very weak
64/46	0.376**	Weak	-0.139^{ns}	Very weak
65/47	0.342*	Weak	-0.132^{ns}	Very weak
66/48	0.303*	Weak	-0.125^{ns}	Very weak
67/49	0.261*	Weak	-0.118^{ns}	Very weak
68/50	0.223^{ns}	Very weak	-0.109^{ns}	Very weak
69/51	0.182^{ns}	Very weak	-0.101^{ns}	Very weak
70/52	0.145^{ns}	Very weak	-0.092^{ns}	Missing
71/53	0.107^{ns}	Very weak	-0.082^{ns}	Missing
72/54	0.068^{ns}	Missing	-0.072^{ns}	Missing
73/55	0.022^{ns}	Missing	-0.060^{ns}	Missing
74/56	-0.023^{ns}	Missing	-0.051^{ns}	Missing
75/57	-0.068^{ns}	Missing	-0.041^{ns}	Missing
76/58	-0.111^{ns}	Very weak	-0.028^{ns}	Missing
77/59	-0.153^{ns}	Very weak	-0.013^{ns}	Missing
78/60	-0.191^{ns}	Very weak	0.002^{ns}	Missing
79/61	-0.227^{ns}	Very weak	0.014^{ns}	Missing

Correlation coefficients showed that the association between age of laying hens, and the daily feed consumption from WA32/14 to WA48/30 is complete, from WA49/31 to WA53/35 very strong, from WA54/36 to WA60/42 strong, from WA61/43 to WA63/45 medium strength, and from the WA64/46 to WA79/61 is very weak, weak or missing. The phenotypic correlation from WA32/14 to WA67/49 is positive and statistically significant, and up to WA62/44 it is statistically very highly significant ($p<0.001$), in WA64/45 and WA63/46 it is highly significant ($r<0.01$), and at the 65th, 66th and 67th week of age of laying hen

correlation is significant ($r<0.05$). From WA68/50 to WA73/55 correlation is a positive, but not statistically significant, while from WA74/56 correlation is negative and not statistically significant. When it comes to phenotypic correlation coefficient between the age of laying hens, and feed consumption per produced egg, a connection up to WA34/16 was negative and highly significant ($r<0.01$), from WA35/17 to WA41/23 was negative and statistically significant ($p<0.05$), while from WA42/24 to WA79/61 was negative, but without statistical significance ($p>0.05$), table 2. Similar results in her work noted Pandurević Tatjana (2011), who has found negative correlation between the age of laying hens hybrid Lohmann Brown and feed consumption per produced egg from 22nd to 53rd week of production, as the correlation connection was determined between the age of laying hens and daily feed consumption, was positive and significant at the level of the $R<0.05$; $R<0.01$ and $R<0.001$.

Conclusions

When it comes to the total consumption of feed per laying hen, we can see a constant increase in the feed consumption until WA43, after which comes to the phased reduction of feed consumption until the end of the production (WA79), with three oscillations in the 60th, 61st and 64th week of age of laying hen, when we noticed a slight increase in feed consumption compared to previous week. During the experiment, an average 5.88 eggs were produced per laying hen a week, while it was spent on average 134.18 grams of feed for producing one egg. Average daily feed consumption per laying hen was 110.07 g, while it was spent an average of 770.46 g of feed per hen a week. By the end of 72nd week of age of laying hens (the established period up to when the commercial flocks in the production of eggs are held) it was produced an average of 5.99 eggs per laying hen a week, while the average were spent of feed was 132.79 g for one egg. It was spent on average 775.20 g of feed per laying hen a week, while average daily feed consumption per laying hen stood at 110.74 g, which is below the values declared as technology standard of examined hybrid. Correlation coefficients showed that the association between age of laying hens, and the daily feed consumption to WA48 is complete, from WA49 to WA53 very strong, from WA54 to WA60 strong, from WA61 to WA63 medium strength, and from the WA64 to WA79 is very weak, weak or missing. The phenotypic correlation to WA67 is positive and statistically significant ($p<0.001$; $p<0.01$; $p<0.05$), from WA68 to WA73 is positive, but not statistically significant, while from WA74 is negative and not statistically significant. When it comes to phenotypic correlation coefficient between the age of laying hens, and feed consumption per produced egg, a connection up to WA34 was negative and highly significant ($r<0.01$), from WA35 to WA41 was negative and statistically significant ($p<0.05$), while from WA42 to WA79 was negative, but without statistical significance ($p>0.05$).

By calculating the phenotypic correlation connection between the age of laying hens, and the daily food consumption per laying hen and per produced egg, it can be concluded that in the present case it is justified to use laying hen in the production of eggs for consumption for 61 weeks. Viewed as a whole, it can be concluded that the analysed commercial flock of light line hybrids Lohmann Brown, in most tracked production indicators achieved satisfactory results whose values are mostly within the limits of the values to the technological standards predicted by selector.

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THE INFLUENCE OF SILAGE DIETS ON THE OF FATTY ACID CONTENT IN MILK FAT

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Abstract

The overview of the investigations with influence of diets with silage on the content and ratio of fatty acids in milk fat was given in this paper. Because of the high significance of animal fats on human health, during the recent decades the attention has been given to the content and ratio of fatty acids in milk fat. Milk fat is especially important because it is obtained without animal sacrifice. Among other factors (breed, lactation phase, gravidity, seasonal variations) the significant influence on the milk fat content can be achieved with the diet, particularly with the ratio of forage and concentrate feeds, and also with the physical effectiveness of dietary fiber. The most favorable ratio and highest content of polyunsaturated fatty acids in milk fat can be fulfilled when animals are fed on pasture or with green forages. However, such feeding regime is limiting the genetic potentials in high yielding breeds and that is the reason why diets are mostly composed with conserved and concentrate feeds. Various types of silages are particularly important for economical milk production, but they have different influence on the content and ratio of fatty acids in milk fat (depending on plant species and vegetation phase). Maize silage, which is most often used in our country, produces increased content of saturated (and undesirable) fatty acids, to a greater extent than any other silage type.

Key words: *milk fat, fatty acids, feeding, silage.*

Introduction

For thousands of years milk and dairy products are important food for people around the globe. They are obtained from living animals, mostly ruminants, which utilize only plant feedstuffs. Also, milk and dairy products are important source of fat and fatty acids in human nutrition, forming 18-24% of total fat in average diet, 30-40% of total saturated fatty acids and 20-25% of total trans- fatty acids (Henderson et al., 2003). During the past decades the positive picture about nutritive value of milk fat (MT) was tampered because of apparent link of all fats with coronary diseases, However, some fatty acids (FA) have very favorable influence on health, particularly conjugated linoleic acid (CLA) (Dhankhar et al., 2016; Đorđević et al., 2020). There are several factors that influence milk fat content and composition such as breed, lactation phase, ratio of concentrate and forage feeds in the ration, physical effectivity of dietary fiber.

Intensive and economical milk production is based on the use of significant amounts of silage throughout the year. Silage has some advantages compared to other forages (fresh or conserved) and that is why it is almost compulsory component of stall feeding systems in cattle. Because of that this paper is dedicated to the influence of maize (corn) silage on milk production, amount of milk fat and content of certain fatty acids.

The use of silage in dairy cow feeding

Cattle feeding, both for meat or dairy production in countries with developed animal production is mostly based on conserved forages (hay, haylage and silage), combined with concentrates all through the year. That way the variability in the diet is reduced, because feed changes during the vegetation are avoided, and the influence of weather when green mass is used directly (cut or as pasture). However, the most variable part of the diet is hay as conserved feed (because weather conditions have high influence on its quality) and can be quite expensive part of the ration, which is why may farmers chose to replace it with silage. In the world the most common silages are made of grass, and as the second comes maize, while silages made of legumes and various by products in crop production are less significant (Wilkinson and Toivonen, 2003). In Serbia the maize silage is dominant, considering the area where this plant is produced, its nutritive value and yields (Đorđević et al., 2012b,c; 2016). Considering that hot summers became more frequent and that it decreased maize yields, some other silage plants became actual (sorghum, sudangrass, triticale, mixes of annual legumes and cereals, beet pulp, brewers grains etc.) (Đorđević et al., 2018b, 2018c; 2019). Whole plant maize silage is a high energy component of the diet, while proteins are provided with lucerne hay and various meals and cakes. As a protein source lucerne silage is becoming increasingly popular, also annual legumes mixed with cereals, and recently the early harvested cereal plans (Đorđević et al., 2012a).

Changes in fatty acid content in milk fat with the silage use

Several factors influence nutritive value in the whole plant maize silage, among them the most important is the choice of hybrid and vegetation phase. Within hybrids the selection is focused on digestibility (Đorđević et al., 2018a). If the maize is ensiled early (DM<250 gkg^{-1}), the ratio of starch/NDF is not favorable, which results in lower DM intake and therefore poorer production of milk and milk fat. While maize maturing the absolute amount of NDF is increased in leaves and stems, but relatively it is decreased because the amount of starch (in grains) is increasing in the whole plant (Table 1). The highest yield of milk and milk proteins is achieved with silages containing 300-350 gkg^{-1} DM, after which it is decreasing.

In spite of the relatively low fat content in maize silage, since it is a large part of the diet it is influencing the total amount of ingested fat and FA. Khan et al. (2012) showed that with the increase of the amount of maize silage in the diet based on grass silage there is increase in FA with medium sized carbon chain (C6:0 to C14:0) and C18:2n-6, and decrease in concentration of C16:0, C18:0 and C18:3n-3. The most important result of the grass silage replacement with maize silage is decrease of n-3 polyunsaturated fatty acids (PUFA) and increase in n-6 PUFA, which is associated with higher n-6/n-3 PUFA ratio. The increase of content of short and medium long carbon chain is the result of higher amount of starch and lower NDF content in maize silage. Higher amount of starch affords higher synthesis of volatile fatty acids (VFA) (acetic and β-hydroxybutyric), which are used for *de novo* synthesis of saturated FA (<16:0) in the udder. Han et al. (2014) in their experiment showed that with the change in the dry matter intake from forages and concentrates (from 60:40 to 40:60) the decrease of total FA occurs, and content of C18: 0 and C18: 3 ($p < 0.01$) in MF, while total FA content (<16C) was increased ($p < 0.05$).

In several investigations it was shown that pasture and green forages, and some types of silage (grass) have key role in the modification of milk fat (Rego et al., 2016; Đorđević et al., 2017; Radonjić et al., 2019). The mentioned feeds (depending on botanical composition and phenological phase) are source of α-linoleic acid (ALA), which is a precursor for CLA. This acid is present in membrane glycolipids in maize, but in much lower amounts than in grass

silage, which can be seen in Table 2. During maturation membrane glycolipids are oxidized with plant lypoxigenasas producing the rapid decrease in FA amounts in chloroplasts, especially C18: 3n-3 (Table 2).

Table 1. Effect of harvest maturity on chemical composition and total tract digestibility of dietary nutrients of maize silage (Khan et al., 2014)

	Harvest maturity			
	Very wet	Wet	Normal	Dry
Chemical composition (gkg^{-1} DM)				
Dry matter (gkg^{-1})	229±19.6	291±10.0	331±14.1	391±38.9
Crude protein	87.4±15.9	74.7±8.41	73.2±9.24	71.8±7.95
Crude fat	24.0±1.73	34.4±5.12	34.8±3.67	34.4±4.43
Starch	134±79.6	301±41.3	339±32.5	374±63.4
NDF	545±50.3	421±45.5	386±47.4	377±52.2
ADF	290±33.5	232±23.1	213±31.3	207±24.8
Fatty acids (gkg^{-1} DM)				
C16:0	-	2.69±0.71	2.95±0.59	3.12±0.65
C18:1	-	3.72±1.21	4.27±1.19	5.19±1.42
C18:2	-	8.98±3.69	9.98±1.95	11.7±2.76
C18:3	-	1.32±0.539	1.14±0.39	0.82±0.305
Total tract digestibility (gkg^{-1} DM)				
Dry matter	653±73.0	-	602±78.3	571±99.1
Organic matter	714±8.14	-	691±37.1	652±57.0
Starch	988±7.21	-	979±25.3	966±44.2
Crude protein	887±11.1	-	826±39.4	804±59.0
NDF	523±41.0	-	444±66.1	388±68.3

Apart from that, cows have higher ruminal pH on pasture, which is favorable for cellulolytic bacteria activity and they are main producers of CLA and vaccenic acid (Mendoza et al., 2016). All silages and the use of increased amounts of concentrates decrease the ruminal pH value. This is one of the reasons why it is necessary to control ruminal pH value in intensively fed cows, using maximal amounts of forages with maximal quality, or with the use of buffers like sodium bicarbonate (which is inferior solution).

Although there are many investigations which show similar results, there are also those where maize silage had positive influence on PUFA and CLA content in milk. According to Shingfield et al. (2005), maize silage combined with low amounts of concentrate (≤350 gkg^{-1}), results in the increase of CLA (probably because it provides more NDF), while according to Sterk et al. (2011) there was depression in MF content with the increase of concentrates in the diet.

Oliveira et al. (2012) showed that with the addition of extruded soybeans in the diets based on maize silage the decrease in short chain FA and palmitic acid and increase of C18: 2 cis-9 trans-11 (CLA) content. This shows that heat processed soybeans can be "corrector" of FA in the milk fat, and at the same time a source of undegradable protein.

Table 2. FA composition of forage ingredients (Khan et al., 2012)

Parameters	Corn silage				Grass silage	Soybean meal
DM, gkg^{-1}	319	324	361	387	286	879
Fatty acids, gkg^{-1} DM						
C8:0	-	-	-	-	-	-
C10:0	-	-	-	-	-	-
C12:0	0.05	0.04	0.05	0.03	0.10	0.35
C14:0	0.08	0.12	0.18	0.12	0.09	0.15
C16:0	2.99	3.01	2.89	2.76	2.86	3.72
C16:1	0.05	0.04	0.07	0.12	0.11	0.03
C18:0	0.46	0.47	0.47	0.48	0.22	0.08
C18:1*cis-9*	4.45	4.45	4.33	4.35	0.31	3.56
C18:2n-6	12.2	12.4	11.9	11,9	2.35	11.6
C18:3n-3	1.56	1.32	0.78	0.58	10.4	1.50
C20:0	0.12	0.12	0.13	0.14	0.05	0.06
C20:1	0.05	0.05	0.05	0.04	-	0.03
C22:0	0.06	0.06	0.06	0.07	0.11	0.09
C24:0	0.10	0.16	0.14	0.11	0.07	0.05
Total PUFA	13.8	13.7	12.7	12.5	12.7	13.9
Total FA	22.5	22.4	21.6	20.9	16.8	22.0

Conclusion

Although maize silage has its deficiencies, as shown in this review, concerning its fatty acid profile, this feed is irreplaceable in the diets for high yield and economic production of both milk and meat. The needed correction of fatty acids the milk fat can be provided combining with other feeds (soybean meal, buffers and other).

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IMPACT OF PARENT FLOCK NUTRITION ON PHEASANT CHICKEN MORTALITY

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Abstract

In the experiment, mortality of white ring pheasant chicken has been researched, on two phasant farms during the period from 2016 to 2018. During non reproductive season, the parents flock in pheasant farm „I" was fed with grain mixture, and in pheasant farm „II" whole diet feed mixture with 18% crude protein (CP) (October/January) and whole diet feed mixture with 20% CP (February – March) were used. But, during reproduction period the parent flocks in both farms used mixture with 22% CP. During rearing period, pheasant chickens in both pheasant farms were fed with whole diet feed with 28% CP in starter phase, and during grower phase they used mixture with 24% CP. In pheasant farm „II" vs. pheasant farm „I" the mortality of pheasant chickens in 15 days age was 2.87% : 2.70%, in age of 16-26 days 0.79% : 0.74%, and in age of 26 to 42 days 0.25% : 0.33%. Not significant lower tottal mortality of pheasant chickens on pheasant farm „II" till 42nd day of age was determined compared to pheasant farm „I", 3.91% vs 3.77%. Based on results it colud be concluded that nutrition with whole diet mixture during non reproductive period allowed the creation of better body reserves, and thus achieving better production results of parents flock (approved in previous experiment) as well as lower (not significant) pheasant chicken mortality.

Key words: *pheasantry, breeding flock, pheasants, nutrition, mortality***.**

Introduction

Pheasant is an attractive game for hunters, so due to the great hunting pressure and insufficient natural production, farm production is carried out, which is the largest in comparison with all other types of hunting game (Beuković and Popović, 2014). According to Popović and Stanković (2009), there are over 30 pheasant farms in Serbia with a capacity of 900,500 one-day-old pheasant chickens. The largest pheasant farm in Serbia was "Ristovaca" in Bac, owned by PE "Vojvodina Sume", whose capacity was about 11,000 individuals in the parent flock (Popovic et al., 2011). In the past four decades, tens of millions of artificially bred pheasants have been released into Serbian hunting grounds. Thanks to its great ecological plasticity, the pheasant has been breeding in controlled farms in Serbia for decades, similar to poultry, and at a certain stage of growth and after appropriate adaptation, it was released into hunting grounds (Đorđević et al., 2012a; 2012b; 2018). Poor survival is evident in such farmed pheasants, combined with significant losses from predators, parasitic infections and loss of fitness (Lanszki, 2005; Ćirović et al., 2008). Sage et al. (2002) state that practice in England is to feed young pheasants after settling in hunting grounds, in order to at least reduce losses. In addition, losses due to the increasing degree of chemicalization and mechanization in agriculture should be emphasized, which have a negative effect on the quantity and choice of natural food, and lead to direct or indirect losses (Draycott et al., 1998;

2005; Hoodless et al., 2001; Swiergosz, 1998). Therefore, it is very important to reduce mortality during breeding on the pheasants themselves, and at the same time reduce production costs (Esen et al., 2010; Nowaczewski et al., 2005). In recent years, pheasant hunting on landfills has become increasingly popular, so farm production of this type of game has become even more current.

There are two basic ways of producing pheasants: a complete process (production of eggs in the parent flock, incubation of eggs and breeding of pheasant chickens until the settlement is inhabited) or just breeding pheasant chickens until the settlement (originating from other pheasants farm). Production parameters in pheasant farms with a complete production process depend on a large number of parameters, the most important of which are: nutrition (breeding flock and later offspring), sex ratio (in the breeding flock), genetic potential, breeding technology, weather conditions, human factor... (Wise, 1994; Kokoszynski et al., 2011; Usturoi, 2008) The success of the pheasant farm business with a complete production process is measured by the number of pheasants chicken per pheasant hen that will be settled in the hunting ground, while the percentage of pheasants that will survive until the hunting season is important for hunting ground users (Pekeč et al., 2008). Due to the importance of this topic for the hunting economy, the paper deals with the study of the influence of the nutrition of the breeding flock in the period outside reproduction (feeding with grain food as a cheaper variant, or complete concentrate mixtures as a more expensive variant), on the survival rate of pheasants in pheasant farm.

Material and methods

The analysis of the production of pheasant chickens was performed in the pheasant farms "Naturall bird ZR" (pheasant farm „I") and "Jagodina" (pheasant farm „II"), in the three-year period, 2016-2018. Both farms are located in Serbia. In the period out of reproduction (October-January), the parent flock on pheasantry „I" was fed with a large mixture of grain, and on farm „II" with a complete mixture with 18% SP. On both farms, in the period before carrying, a complete mixture with 20% SP was used, and in the period of carrying the mixture with 22% SP. Pheasantry „I" does not have an incubator station, but incubates eggs in the pheasantry of the hunting association "Morava" from Ćuprija. In contrast, Pheasantry „II" has its own incubator station, which also performs service incubation of eggs for the hunting association "Crni vrh". On both farms, in the first four weeks of age, the pheasants were fed a mixture with 28% protein, and then a mixture with 24% protein. The death of pheasants was recorded ad examined pheasants groups by the age: up to 14 days of age, from 15 to 25 days of age, from 26 to 42 days, as well as the total death during the rearing period. Testing the significance of the differences of the examined parameters between the observed farms was performed by t-test.

Results and discution

In pheasantry „I", the lowest mortality at the age of pheasants up to 14 days was recorded in 2018 (2.1%), and in pheasantry „II" in 2016 (2.35). The lowest percentage of pheasant deaths at the age of 15 to 25 days was recorded on both farms in 2017. The highest mortality rate in the last examined phase (from 26 to 42 days) on farm „I" was determined in 2018, and on farm „II" in 2016. The highest total mortality for the period up to 42 days of age on farm „I" was determined in 2016 (4.98%), and on farm „II" in 2018 (4.05), while the average mortality for this period on farm „I" was 3, 91%, and on farm „II" 3.77%. The highest mortality on both examined farms was recorded in the first period of rearing, when pheasants are most sensitive, in order to decrease in each subsequent phase of upbringing.

Table 1. Results of monitoring pheasant farms by rearing period during the years

Year	Total hatched	% mortality up to 14 days	Survived on the 14th day	% mortality from 15 to 25 day	Survived on the 25th day	% mortality from 26 to 42 day	Survived on the 42nd day	% Total mortality
Pheasant farm I								
2016	9,578	3.6	9,376	1.2	9,287	0.18	9,276	4.98
2017	8,376	2.9	8,133	0.46	8,099	0.21	8,082	3.57
2018	7,767	2.1	7,642	0.72	7,606	0.36	7,578	3.18
Pheasant farm II								
2016	8,069	2.3	7,980	0.69	7,933	0.41	7,906	3.40
2017	9,779	3.1	9,524	0.43	9,493	0.33	9,476	3.86
2018	10,717	2.7	10,534	1.1	10,453	0.25	10,438	4.05
Farm I, Ø	8,573.67	2.87	8,383.67	0.79	8,330.67	0.25	8,312.00	3.91
Farm II, Ø	9,521.67	2.70	9,346.00	0.74	9,293.00	0.33	9,273.33	3.77
t value	-	0.3533	-	0.1689	-	0.4515	-	0.2421
Significance	-	ns	-	ns	-	ns	-	ns

$t_{0,05}$=2,776; $t_{0,01}$=4,604

Since the pheasants in both farms were fed concentrate with the same protein content, the minimal differences in the percentage of losses on both experimental farm can be attributed to differences in the nutrition of the parent flock, but also to some other factors. According to previous researchers (Đorđević et al., 2020) on pheasantry „II" in relation to pheasantry „I", it was found that significantly annual laying capacity was (53.00: 42.25; $p<0.01$), higher average daily number of eggs per pheasants (0.51: 0.47; $p<0.01$), and a higher average annual number of hatched pheasants per pheasant (29.30: 24.85; $p<0.01$). Based on the cited results, it can be concluded that it will result in a complete game during the period out of reproduction, allowing a large amount of body reserves and achieving better production results of the parent flock. In addition, egg mass largely depends on the diet. Caglaian et al. (2010) found that pheasants of larger mass hatch also from larger eggs ($p<0.001$), which is a good predisposition for their vitality. Ipek and Dikmen (2007) classified eggs by weight into three groups (27.8-29.7; 29.8-31.7 and 31.8-33.7 g) and determined the masses of pheasants in the same order from 19, 5; 21.8 and 22.6 g ($p<0.01$). Beuković (1999) used three mixtures of concentration with 22%, 19% and 16% of crude proteins in the diet of the parents flock of pheasants, where the energy level was the same in all mixtures (11.71 MJ). The weights of pheasants at hatching are 21.87 g, 21.59 g and 21.09 g, ie the highest weight had chickens from the groups of carried feed mixtures with the highest level of protein. In a previous experiment, Djordjevic et al. (2020), on farms I and II, egg ovoscoping is not performed, but it is classified on the basis of weight and shape, which is directly used on the number of hatched pheasants per pheasant.

It is unlikely that the higher average mortality of pheasants on the farm is also a direct matter of poorer nutrition of the parent flock in the period outside reproduction. Namely, the mortality of pheasants depends the lowest on a number of environmental factors, such as temperature, ventilation, lighting, hygiene, etc. According to Jović (1964), the average mortality of pheasants up to an increase of 20 days was 7.92%. Braastad (1986) states that the mortality rate for 16 weeks, including birds died of picking, was 2.0 to 8.7%. Popović and Stanković (2009) showed that in two pheasant farms in Serbia, mortality of pheasants up to

40 days was determined in the first pheasant farms from 8.33 to 13.39% in relation to the others from 3.60 to 4.74%. Djordjevic et al. (2011) states that the mortality rate with pheasants up to the 42nd day of life was less than 3%. According to Djordjevic et al. (2013), even when feeding pheasants mixed with different percentages of protein, no significant differences in average death were found, precisely because of the influence on a large number of other factors.

Conclusion

It can be concluded that differences in the nutritional value of the feed for the parents flock have a significant impact on the production parameters of pheasants, but not on the mortality of pheasants. The success in the business of a pheasant farm with a complete production process (pheasant farm „II") is measured by the number of pheasants per pheasant that will be inhabited in the hunting ground. The percentage of mortality during the rearing of pheasants is certainly important for that number, but it is not crucial, especially if it is less than 3-4%.

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INFLUENCE OF MILK FAT CONTENT ON THE PROPERTIES OF FULL FAT YOGURT

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Abstract

In the last couple of decades, full fat yogurt has been produced and consumed in Serbia not only as a stirred, but also as a Greek-style yogurt. Milk fat (MF) content of these products varies in a wide range from 2.8-3.2% in stirred yogurts to 8-9.7% in Greek-style yogurts. In addition to the physico-chemical characteristics, milk fat has a great influence on the rheological and sensory properties of yogurt. Thus the aim of this work was to investigate the influence of milk fat on the above mentioned yogurt characteristics. As expected, due to significantly higher MF content, higher levels of total solids (17.80-20.00%) were detected in Greek-style yogurts. Futhermore, the highest protein content (6.92%) was also recorded in yogurt with 8% MF. Higher total solids in Greek-style yogurts infulenced stability of casein gel structure so in these samples syneresis was not detected. Also, significantly higher ($p<0.05$) values of viscosity were recorded in Greek-style yoghurts. On the other hand, lower MF content and subsequently lower total solids in stirred yogurts influenced lower viscosity and occurrence of sineresys (12.47-22.09%). The taste had the greatest influence on the overall sensory quality of the yogurt. Greek-style yoghurt samples showed significant difference ($p<0.05$) in terms of average taste rating and pondered mean value. However, although not rated as the best regarding taste, yogurt with 9% MF had the highest percentage of maximum quality - 94.33%. Stirred yoghurt samples did not differ significantly in any parameter of sensory quality and for those samples percentage of maximum quality was in the range 84.94-85.14%.

Key words: *full fat yogurt, viscosity, syneresis, sensory quality*

Introduction

Due to the health benefits of yogurt on the human body, there is a growing trend of yogurt consumption in the world. Therefore, yogurt and fermented beverages are produced in many styles and varieties with different fat contents, flavors and textures (Weerathilake et al., 2014). Stirred yogurt is produced by a well-known technological procedure. However, the quality of milk in different geographical regions varies and production procedures in terms of heat treatment, starter culture, fermentation, cooling and storage of yogurt are different for each dairy plant. In Europe and North America, Greek yogurts are often made with a low fat content and a high protein content which can be achieved by draining, centrifugal separation, ultrafiltration or addition of milk-protein based products. On the other hand, Greek-style yogurts are characterized with high milk fat content and could be obtained from milk standardized to a higher milk fat content than usual (eg about 7%). The product is thick, creamy, white in color with a higher dry matter content compared to ordinary yogurt (23-25% SM) (Tamime and Robinson, 2000). Since there is no standard for Greek yogurt, it can be obtained by different procedures, which in turn leads to variability in the sensory and textural properties of the product (Samaržija, 2015). All of the above indicates different quality of full fat yogurts consumed in Serbia. Considering that milk fat has a great influence on the physico-chemical characteristics, rheological and sensory properties of yogurt, and that in full

fat yogurts milk fat content varies in a wide range, the aim of this work was to investigate the influence of milk fat on the above mentioned characteristics of full fat yogurts available on Serbian market.

Material and method

Full fat yogurt samples present on the Serbian market were collected during the months of May and June and used for the investigation of the influence of milk fat content on the properties of cow's milk yogurt: 9 samples of stirred yogurt (2.8% - 3.2% MF) and 3 samples of Greek-style yogurt (8% - 9.7% MF).

The following methods were used for the analysis of physico-chemical composition of yogurt samples: total solids (Carić et al., 2000), proteins (IDF 20B:1993), fat in yogurt (Carić et al., 2000), titrable acidity (IDF 105:1991), pH value with pH meter (Consort C931). Each analysis was done in triplicate.

Syneresis was determined according to Ramirez-Santiago et al. (2010). For these tests, Laboratory Centrifuges (Ependorf, USA) was used and analyses were done in duplicate.

The viscosity of yogurt samples was determined using a rotary viscometer: Visco Basic + R, (Fungilab, Spain) at constant speed of spindle rotation according to the method described by Vučić et al. (2010). For each yogurt sample test was performed twice and the average viscosity was calculated.

The overall sensory quality was determined by point rating scale method (Radovanović and Popov-Raljić, 2001) by trained judges.

The effect of milk fat content on yogurt characteristics was analyzed through Statistica 6.0 software (Stat Soft. Inc., Tulsa, USA), using analysis of variance (ANOVA). The comparisons of means were performed by t-test, with the level of significance at 0.05.

Results and discussion

All yogurt samples are produced from cow's milk in industrial conditions, originating from different manufacturers. The defined specific process parameters, such as thermal-mechanical and time regimes, as well as the choice of starter cultures, depended on the type of yogurt, milk fat content and manufacturer. All of the above indicates that differences in the physico-chemical composition, certain rheological and sensory properties of the tested samples could be expected.

Physico-chemical characteristics of the investigated full-fat commercial yogurts are presented in Table 1.

Considering physico-chenical characteristics, investigated samples of stirred yogurt differ significantly only in terms of milk fat content ($p<0.05$).

Significant differences in milk fat content were also found in Greek-style yogurts. However, yogurt samples with 9% MF had lower milk fat content than declared, which did not meet the requirements of the legislation on the minimum content of milk fat. As expected, due to significantly higher MF content, Greek-style yogurts had much higher levels of total solids (17.80-20.00%) than stirred yogurt samples. Yogurt samples with 8% MF had the highest content of TSNF (12.19%) and proteins (6.92%). Such high protein content in samples with 8% MF indicates that in its production milk-protein based products were used in order to to strengthen the protein matrix. The highest acidity and pH values were also found in these samples (1.20% l.a. and 4.62, respectively). Observed pH values in these samples are probably a consequence of higher buffer capacity due to higher protein content. Additionaly, these samples presumably had shorter storage period since all yogurts were obtained from the market. Therefore high acidity in Greek-style yogurts with 8% MF could be attributed to the

increased growth of LAB due to the addition of milk-based proteins such as milk protein concentrates or whey protein concentrates (Herrero and Requena, 2006; Vučić et al., 2011).

Table 1. Physico-chemical characteristics of yogurt

Declared fat content (%)	Parameter					
	TS (%)	MF (%)	TSNF (%)	Proteins (%)	Acidity (% l.a.)	pH
Stirred yogurt						
2.8	11.32±1.04^{a}	2.51±0.17^{b}	8.81±1.02^{a}	3.30±0.47^{a}	0.89±0.19^{a}	4.48±0.12^{a}
3.2	11.26±0.28^{a}	3.01±0.23^{a}	8.25±0.13^{a}	3.11±0.12^{a}	0.81±0.07^{a}	4.48±0.10^{a}
Greek yogurt						
8	20.00±0.01^{a}	7.81±0.16^{b}	12.19±0.17^{a}	6.92±0.04^{a}	1.20±0.00^{a}	4.62±0.00^{a}
9	17.80±0.01^{c}	8.03±0.16^{b}	9.77±0.17^{b}	3.99±0.05^{b}	0.87±0.01^{b}	4.57±0.00^{b}
9.7	18.30±0.07^{b}	9.68±0.00^{a}	8.62±0.07^{c}	3.57±0.03^{c}	0.95±0.01^{b}	4.46±0.00^{c}

* TS – total solids, MF – milk fat, TSNF – total solids non fat

Different superscripts within the same column indicate that the means differ significantly ($p<0.05$).

Syneresis is the separation of the liquid phase from the gel, so in yogurt this process is undesirable. It can be explained by the number of interactions between casein and whey proteins. With more bonds, a more crosslinked gel is formed which is characterized by a greater ability to retain water (Lee and Lucey, 2003). Spontaneous separation of whey on the gel surface is the result of instability and rearrangement of the protein network (Lucey and Singh, 1997). Figure 1. shows the mean value of the extracted whey of the of full-fat stirred yogurt samples.

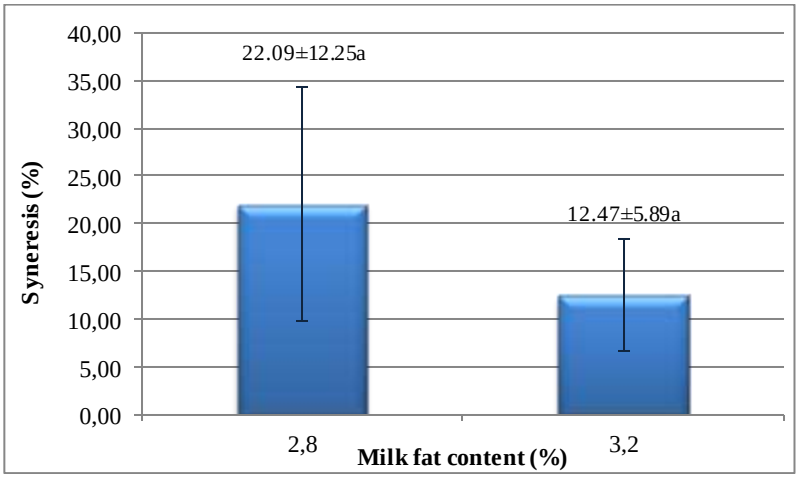


Figure 1. Syneresis of stirred yogurts

*Different superscripts indicate that the means differ significantly ($p<0.05$)

According to Figure 1 milk fat contnet did not influence syneresis of stirred yogurts. Also, since stirred yogurts did not differ significantly regarding TSNF, protein content and pH which are the main factors influencing syneresis, there was no significant differnce among samples regarding expulsion of whey. Unlike stirred yogurt samples, Greek-style full-fat yogurts showed great resistance to whey separation. High TS content in these samples significantly affected stability of the gel structure so syneresis was completely absent.

Viscosity of yogurt, as a significant rheological property is greatly influenced by milk composition, increase of total solids content, heat treatment and homogenization of milk, and applied starter culture (Sodini et al., 2004; Purwandari et al., 2007).

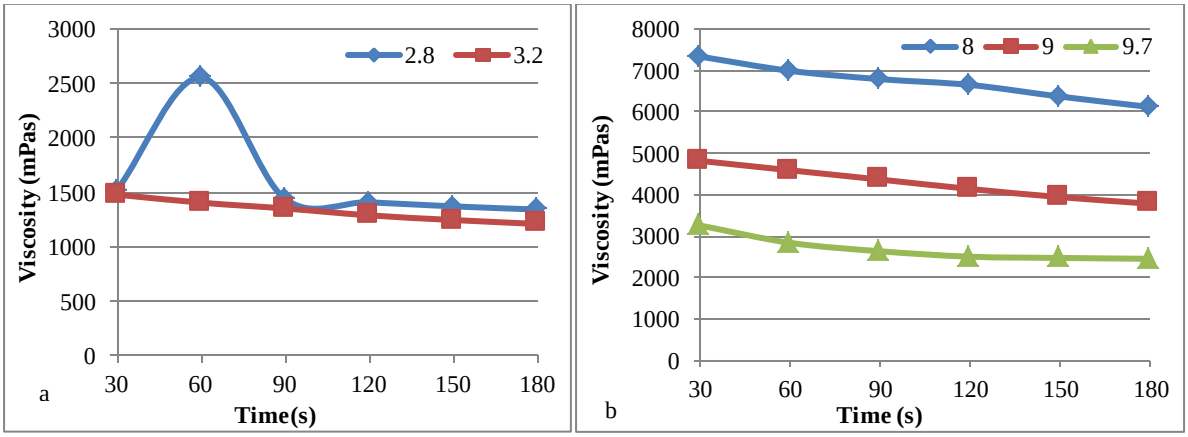


Figure 2. Viscosity of yogurts (a – stirred; b – Greek-style)
* Numbers in Legend indicate declared fat content

Higher milk fat content leads to an increase in the viscosity and higher firmness of yoghurt (De Lorenzi et al., 1995). However, milk fat content did not affect viscosity of investigated stirred yogurts (Figure 2a). Under the influence of the shear force, the viscosity decreases over time, but due to post-incubation operations during which the structure of the gel is broken (Afonso and Maia, 1999) viscosity of stirred yogurts did not significantly change during measurement. The only deviation from thixotropic behavior was observed in samples with 2.8% MF after 120 s, when the viscosity value was 1032.8 mPas higher than the value recorded after 90 s of measurement. Among Greek-style yogurts, significantly higher viscosity was recorded in samples with 8% MF which is mainly the result of the highest levels of TSNF, especially proteins. Also, there was no significant change in viscosity during measurment. On the other hand, in yogurts with 9% and 9.7% MF significantly lower viscosity was observed over time. Contrary to expectations, among Greek-style yogurts, samples with 9.7% MF had the lowest viscosity which could be explained by lower protein content.

The sensory characteristics of yogurt are influenced by the type and quality of milk, technological production process, type and activity of starter culture (Tamime i Robinson, 2000; Güler i Park, 2011). The average score for each sensory attribute and the overall preference of the samples are shown in Figure 3. (a, b).

As presented in Figure 3a, no significant difference in any of the tested properties of sensory quality was found between stirred yogurt samples. Percentage of maximum quality for those samples was in the range 84.94-85.14%. Significant difference ($p<0.05$) in terms of average taste rating and pondered mean value was established in Greek-style yogurts (Figure 3b). Regarding taste, samples of yogurt with 8% MF (4.83) were evaluated as the best. However, due to all the other sensory atributes yogurt with 9% MF was evaluated as the best among all investigated yogurts with the highest pondered mean value (4.72).

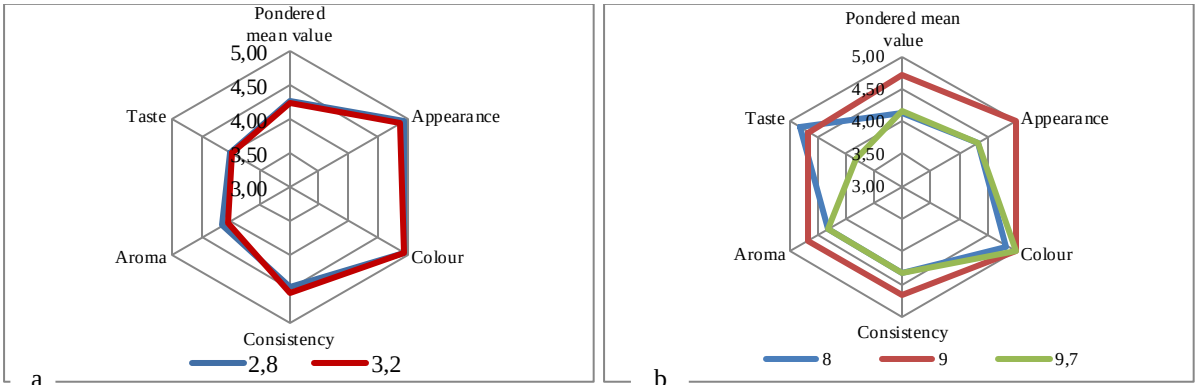


Figure 3. Sensory evaluation of yogurt (a – stirred; b – Greek-style)
* Numbers in Legend indicate declared fat content

Conclusion

Two varieties of full fat yogurts (stirred and Greek-style) are present on Serbian market. Milk fat content did not affect physico-chemical, rheological and sensory characteristics of stirred yogurts. Due to high total solids content (higher milk fat and protein content) much better rheological characteristics (significantly higher viscosity and abscence of syneresis) had Greek-style yogurts. The highest viscosity was found in samples with 8% MF, but this is a consequence of a firmer gel due to a 1.77-1.94 times higher protein content. Based on sensory evaluation, Greek-style yogurt with 9% MF had the highest sensory scores and therefore it was evaluated as the most acceptable to the consumers.

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EFFECT OF SPACE ALLOWANCE ON BEEF CATTLE PERFORMANCE AND HEALTH

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Abstract

A low space availability for finishing cattle could compromise animals' health, welfare and growth. The aim of this study was to investigate on how space allowance can affect health and growth of finishing Charolais bulls housed in an indoor facility. The trial involved 108 bulls with an initial individual space of 3.50 or 4.37 m^2 per animal, for the low (LSA) or the high space allowance group (HSA), respectively. For each animal the health conditions were collected daily and the average daily gain (ADG) was measured throughout the trial. Data on ADG were analyzed through a mixed ANOVA model, whereas data on symptoms and treatments were analyzed through the non-parametric Kruskal-Wallis test. Because of an unexpected culling rate due to lameness, the space allowance increased throughout the trial up to 4.07 and 4.62 m^2 per animal for LSA and HSA, respectively. Average daily gain was not affected by space allowance and was on average 1.28 Kg/day, but LSA led to a higher number of treatments per sick animal (1.23 vs. 2.60, P=0.011). This study shows how higher space allowance in finishing bulls can help in improving animal health in indoor systems.

Keywords: *Charolais, Lameness, Stocking density, Growth.*

Introduction

In Italy, beef cattle production represents an important percentage of the meat production industry with the 10.4%. of the EU-28 total beef meat yield (Eurostat, 2019). Italian beef farms can be roughly divided in a first group, consisting in the intensive livestock systems, 70-75% of which are situated in North-Eastern Italy (Cozzi, 2007) and in a second group including the extensive production systems, located in the North-western and in the Central-Southern Italy. Italian beef farmers belonging to the first group are strictly dependent on imported animals from abroad, especially from France and their fattening units have a capacity ranging from 200 to over 2000 heads. Cattle arrive at the age of 10–14 months and initial body weights of 300–400 kg. Most common genotypes are Charolaise and Limousine, followed by crossings and other French local breeds.

Upon their arrival bulls are grouped in multiple pens and kept in indoor facilities for about 7 months where they are generally fed *ad libitum* a high concentrate diet, provided as total mixed ration (TMR) (Marchesini et al., 2018a). The housing design mostly adopted is the concrete slatted floor system, which on the one hand has the advantage of reducing labor, saving costs of bedding materials and preventing the pollution risks (Scahaw, 2001), but on the other has been proved to increase the risk of early culling rate mainly due to lameness and musculoskeletal disorders (Gygax et al., 2007). Moreover, indoor slatted floor facilities are often associated with a small space allowance per animal (Daelemans and Maton, 1987) which is another critical issue for animal welfare.

An insufficient space for lying down may reduce resting time and increase frequency of disturbance episodes of the lying animals (Gygax et al., 2007) and together with the reduction in feeding space at manger, leads to competitive behavior and social tension between pen mates (Morrison et al., 2003), that may worsen average daily weight gain (ADG) and feed

conversion ratio (FCR). A narrow space allowance has been shown to alter normal feeding behavior and, on slatted floors, increase the incidence of tail tip lesions (Andersen et al., 1997). In a French survey (Beranger, 1986) it was reported that the mortality of bulls decreased when the air volume (Table 1) and space allowance (Table 2) increased. Transmission of respiratory diseases, in fact, is favored by close contacts with other animals or with fomite (Tennant et al., 2014). Although Ingvartsen and Andersen (1993) reported that a space allowance of less than 4.7 m^2 per animal on slatted floors, weighing between 250 to 500 kg, decreased ADG, there are no European Regulations that impose a minimum floor space for fattening cattle, but only a recommendation that animals should be provided with a minimum space allowance of 3 m^2/head for animals until 500 kg, plus 0.5 m^2 or minus 0.5 m^2 for each 100 kg difference expected between 400 kg and 800 kg (SCAHAW, 2001).
The aim of this study was to find out whether or not space allowance can affect health and growth of finishing Charolais bulls housed in an indoor facility during the growing and finishing phases.

Materials and methods

The research was endorsed by OPBA (Organismo Preposto al Benessere Animale-Animal Welfare at ISS committee) of Padova University and conducted from July to August 2018.
One hundred and eight Charolais bulls weighing on average 424 ± 30 kg, after 45 days of backgrounding phase, were split into two groups, homogenous in weight and housed for 131 days in 12 contiguous pens at 10 and 8 animals per pen alternatively, corresponding to 3.50 square meters per animal for the low space allowance group (LSA) and 4.37 square meters per animal for the high space allowance group (HSA), respectively.
Because of the onset of a severe lameness problem and the consequent severe culling rate, the space allowance within the pens changed over time and we had to attribute the LSA class to those pens with more than 8 animals per pen, whereas we maintained HSA class for pens with 8 or less animals per pen. The ration was the same for both groups and included maize meal, maize silage, pressed beet pulps, wheat bran, straw, soybean meal and a protein/vitamin/mineral mix. From the first to the second period adjustments were made to the ration, passing from 47.8% of dry matter (DM), 13.7% DM of crude protein (CP) and 28.9% DM of starch, to 47.3% of DM, 13.3% DM of CP and 34.2% DM of starch. All the animals were equipped with SCR collars (HRLDn Tag; SCR Engineers) to estimate their daily activity and rumination. To calculate ADG and FCR, the bulls were weighed at day 1, 70 and 131 of the trial and dry matter intake per pen was measured daily by the difference between TMR distributed and 24 h leftovers. Bulls' health status was verified daily and regularly recorded together with data of treatments, culled animals and fatal events.
Statistical analysis was performed using SAS software (release 9.4; SAS Institute Inc., Cary, NC). Upon verifying normal distribution data were analyzed using a mixed ANOVA model. Pen within stocking density was chosen as random effect, whereas period, class of space allowance and their interaction were used as fixed effects. The non-parametric Kruskal-Wallis, Z and Exact Fisher tests were chosen to compare the number of treatments per animal, the percentage of animals treated or culled and the culling rate between HSA and LSA.

Results and discussion

Against our expectation, space allowance did not affect bulls' ADG, DMI and FCR (Table 1), whereas as expected, bulls in the second period showed a significant increase in DMI ($P<0.001$). The interaction period × space allowance was never significant.

Table 1. Effect of period (P1 and P2) and space allowance (LSA and HSA) on dry matter intake (DMI), average daily gain (ADG), feed conversion ratio (FCR), daily activity and rumination.

Parameter	**Period**		**Space allowance**			***P*-value**	
	P1	P2	LD	HD	SEM	Period	Space allowance
ADG (kg/d)	1.33	1.25	1.32	1.24	0.110	0.418	0.466
DMI (kg/d)	9.88	10.6	10.1	10.6	0.305	<0.001	0.256
FCR	7.84	8.62	7.99	8.82	0.720	0.208	0.276
Daily activity (bit)	373	378	384	368	5.55	0.355	0.015
Daily rumination (min)	342	299	320	321	11.6	<0.001	0.936

SEM = standard error of means.

Marquis et al. (1991) showed that in condition of very low space allowance (1.5 m^2 per head), even an increment of 0.5 m^2 could lead to an increase of 300 g of ADG. This result, as suggested by Mogensen et al. (1997) was likely due to shorter lying and feeding times available for the animals with the lowest space allowance, leading to a reduced daily gain. In our study, the lack of difference in performance between LSA and HSA bulls is probably to be associated to many factors such as a less severe space allowance, which in LSA at the starting conditions was 3.5 m^2, and the culling rate at the end of the trial that increased the space allowance up to 4.07 m^2 and 4.62 m^2 in LSA and HSA, and reduced the difference between them to 0.55 m^2. Moreover, severe lameness has probably compromised DMI and animals' growth, making difficult to compare animals' performance.

With regard to SCR collars measures (Table 1), daily activity of HSA animals was higher compared with LSA, meaning that giving more space encourages animals' activities, whereas rumination did not change.

Throughout the trial 35 animals needed to be treated: 26 bulls showed severe lameness, 4 suffered from bovine respiratory disease (BRD), and 5 had miscellaneous ailments. After recovery, 11 out of the 26 lame animals and 1 out of the 4 animals with BRD, showed a relapse, needing a further pharmacological intervention.

Throughout the trial 16 animals, (4 HSA and 12 LSA), had to be culled for lameness (Table 2).

Table 2. Effect of space allowance on animals' health status

	Space allowance		*P*-value
	HSA	LSA	
Treated bulls (%)[1]	14.7	21.5	0.271
Treated bulls for lameness (%)[1]	12.7	18.7	0.319
Early culled bulls (%)[2]	8.33	20	0.108
Pen data[3]			
Prevalence of total diseases	0.24	0.15	0.432
Prevalence of lameness	0.21	0.14	0.431
Average number of treatments per sick animal	1.23	2.60	0.011
Average number of treatments per lame animal	1.01	2.30	0.032

[1] Percentage of total bulls treated: Z test
[2] Percentage of total bulls culled: Fisher's exact test
[3] Average data per pen: Kruskal-Wallis test

Space allowance, in this condition, does not seem to significantly affect the percentage of total bulls treated for lameness or other disorders, the prevalence of diseases and lameness within pens or the culling rate. However, animals raised at higher density showed the need of

more pharmacological treatments to fully recover from lameness or other diseases, due to the onset of relapses after the first medical intervention. These outcomes match with those of other authors (Callan and Garry, 2002), who found that a lower space allowance enhances contact between bulls, pathogens transmissions and increases animal stress weakening the immune system.

Conclusion

The higher space allowance led to a lower number of relapses due to lameness or other diseases, thus facilitating the maintenance of animals' health. The outcomes of this research suggest that increasing space allowance in finishing bulls is worthwhile for reducing the impact of lameness and other diseases.

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EFFECT OF PROTEASE ADDED IN FOOD ON CHICKEN CARCASE QUALITY

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Abstract

This study evaluates the effect of protease-supplemented diets containing different crude protein levels and sex on the weight and percent yields of individual meat classes (class I meat: breast, drumsticks and thighs; class II meat: wings, and class III meat – back and pelvis) in Master Gris broilers (medium-growing strains). Chickens were fed maize-and-soybean-based diets. The fattening period lasted 49 days. Broilers were allocated to 3 dietary treatment groups: group C (standard diet, 0% protease), group E-I (0.2% protease, crude protein level reduced by 4% compared to C) and group E-II (0.3% protease, 6% reduction in crude protein compared to C). Results showed that no significant differences were observed in the weight of class I and class II meat between dietary treatments ($P>0.05$). Reduction of crude protein by 4% in diets supplemented with 0.2% protease affected the weight class III meat in male chickens ($P<0.05$). The feeding treatments did not influence the percentage of class I and class III ($P>0.05$), while significant differences were observed in percentage of class II meat (between male broilers in C and E-I groups, $P<0.05$). The effect of sex was significant on both weight and percentage of all three meat categories ($P<0.05$).

Keywords: *broilers, protease, sex, class meat.*

Introduction

Protein is the second major nutrient (after energy) and the most expensive in the broiler diet. The protein sources in modern poultry diets are mostly derived from soybean products.
The environmental impact from nitrogen and phosphorus that comes from undigested proteins and other excreted substances in the poultry manure (Gerber et al., 2015) has led to the idea of using supplemental exogenous enzymes like proteases in poultry diets.
Most commercial enzyme products currently available have more than one enzyme activity, whereas fewer products have only one substrate specificity. Enzyme blends are products having more than one enzyme and are either combinations of mono-component enzymes, generated by mixing enzymes targeting defined feed substrate matrixes, or fermentation products from wild-type strains of microorganisms expressing a broad spectrum of enzyme activities (Frietas et al., 2011).
Supplementation of the diet with enzymes which treat one or more of the factors limiting digestion enhances more complete protein digestion and more efficient growth (Kamel et al., 2015). Protease supplementation in diets for broilers improves the efficiency in utilizing vegetable and animal protein (Vieira et al., 2016) and improves protein digestibility and decreases synthesis of endogenous enzymes, resulting in higher availability of amino acids for protein deposition and minimizing protein waste (Kamel et al., 2015).
Many researchers (Dosković et al., 2017; Xu et al., 2017; Law et al., 2018) have studied the effect of protease supplementation of broiler diets on meat quality.
According to relatively rare research on non-industrial broiler hybrids, a medium hybrid Master Gris was selected for testing.

Thus, the objective of the present study was to evaluate the effect of reduction in the amounts of crude protein in corn and soybean meal-based diets and supplemented with protease enzyme on carcass characteristics (meat class) of medium-growing Master Gris chickens.

Materials and Methods

In the experiment, 300 day-old medium-growing Master Gris broilers were randomly assigned to three groups, each comprising 100 birds. Feed and water were available *ad libitum* throughout the experimental period. The experiment was performed in 2011.

Dietary treatments

The feeding trial was conducted over 49 days through starter (the first 3 weeks), grower (22-42 days) and finisher (42-49 days) stages. The following feeding treatments were used: control - C (control diet), experimental group E-I (crude protein levels reduced by 4% than in the control diet, 0.2% protease supplementation - Ronozyme ProAct) and experimental group E-II (crude protein levels reduced by 6% than in the C diet, 0.3 % protease supplementation - Ronozyme ProAct). Feed formulation was in powdered form. The diets were formulated based on corn and soybean products (Table 1).

Table 1. Composition of experimental diets for each rearing period chickens[1]

Ingredient, %	Starter stage (1 to 21 d)			Grower stage (22 to 42 d)			Finisher stage (43 to 49 d)		
Treatments	C	E-1	E-2	C	E-1	E-2	C	E-1	E-2
Corn	52.49	54.92	56.26	63.15	65.28	66.34	68.62	70.60	71.59
Soybean meal	22.24	19.79	18.44	13.00	10.85	9.78	9.10	7.10	6.10
Soybean groats	18.50	18.50	18.50	17.00	17.00	17.00	15.40	15.40	15.40
Feeding yeast	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
L-Lysine (78%)	0.10	0.10	0.10	0.20	0.20	0.20	0.23	0.23	0.23
DL-Methionine (99%)	0.22	0.22	0.22	0.30	0.30	0.30	0.30	0.30	0.30
Limestone	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Monocalcium phosphate	1.30	1.30	1.30	1.20	1.20	1.20	1.20	1.20	1.20
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Calcium formiate (30.5%)	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Captex T	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Premix	1	1	1	1	1	1	1	1	1
Protease	0.00	0.20	0.30	0.00	0.20	0.30	0.00	0.20	0.30
Calculated composition									
ME, kcal/kg	3.081	3.100	3.112	3.157	3.174	3.183	3.181	3.198	3.207
Crude proteins, %	22.59	21.72	21.24	18.99	18.22	17.84	17.16	16.45	16.09

[1] Treatments: C-control group, standard broiler diet, without protease; E-I- broilers fed a diet with a 4% reduction in crude protein level as compared to the control group, and 0.2% protease supplementation; E-II broilers fed a diet with a 6% reduction in crude protein level as compared to the control group, and 0.3% protease supplementation.

Data collection

At the end of the experimental period i.e. at 49 days, a total of 12 male and 12 female broilers were randomly selected from each group of birds (3 male and 3 female broilers per replication). The selected chickens after slaughter, were dissected into primal cuts - breast, drumsticks, thighs, wings, back, pelvis (according to the Commission Regulation (EC) No. 543/2008) and meat class (class I meat: breast, drumsticks and thighs; class II meat: wings, and class III meat

- back and pelvis) were weighed. Based on the weights of meat class the proportions of meat class were determined.

Statistical analysis

The significance of differences for carcass quality parameters (weight and percent yields of individual meat classes) was tested by analysis of variance i.e. in a two-factor 3x2 design (3 feeding treatments and 2 sexes).

Meat class parameters were statistically evaluated using analysis of variance, F-test and LSD test (Stat Soft Inc Statistica For Windows. Version 7.0., 2006), at $P<0.05$.

Results and Discussion

Weight of individual meat classes are presented in Table 2.

Table 2. Weight of different classes of chicken meat in the dressed carcass on days 49, gr

Treatment			Class I (breast, thighs, drumsticks)	Class II (wings)	Class III (back, pelvis)
Groups	Sex				
C (no protease)	Male	X̄	1182.74[a]	239.74[a]	465.77[a]
		Sd	46.79	7.75	21.51
	Female	X̄	1028.83b	203.75[b]	402.47[c]
		Sd	43.90	4.77	17.61
E-I (0.2% protease)	Male	X̄	1144.38[a]	237.31[a]	434.09[b]
		Sd	85.52	10.53	25.66
	Female	X̄	991.26[b]	201.02[b]	385.91[c]
		Sd	33.31	6.05	15.37
E-II (0.3% protease)	Male	X̄	1155.44[a]	239.37[a]	452.29[ab]
		Sd	61.94	10.17	29.09
	Female	X̄	1000.20[b]	199.00[b]	391.92[c]
		Sd	66.69	12.16	28.98
p-value					
Source of variation					
Protease			0.117	0.581	0.008
Sex			0.001	0.001	0.001
Protease x sex			0.998	0.690	0.566

X̄ -Average, Sd - Standard deviation

[a-b] Means within columns with different superscripts differ significantly ($P<0.05$)

Analysis of the data in Table 2. showed that no significant differences were observed in the weight of class I and class II meat between dietary treatments ($P>0.05$), but there were differences in the class III meat weight. Namely, control males had higher weight class III meat compared to E-I male broilers ($P<0.05$). Also, the data in Table 2 showed a significant effect of broiler sex on the weight of the all three meat categories ($P<0.05$), because male chickens had a higher mass of all three classes of meat compared to female chickens, which was due primarily to lower body weight at slaughter. Similarly, some other authors (Blagojević et al., 2009; Dosković et al., 2016) have reported for the effect of broiler sex on some parameters of carcass quality, regardless of age.

Table 3. The percentage of different classes of chicken meat in the dressed carcass on days 49, %

Treatment			Class I (breast, thighs, drumsticks)	Class II (wings)	Class III (back, pelvis)
Groups	Sex				
C (no protease)	Male	X̄	60.50^{ab}	12.27^{b}	23.82
		Sd	0.70	0.43	0.42
	Female	X̄	60.00^{b}	11.89^{c}	23.48
		Sd	1.36	0.39	0.85
E-I (0.2% protease)	Male	X̄	60.91^{a}	12.65^{a}	23.13
		Sd	1.02	0.32	0.72
	Female	X̄	60.06^{ab}	12.18^{bc}	23.38
		Sd	0.77	0.37	0.68
E-II (0.3% protease)	Male	X̄	60.40^{ab}	12.52^{ab}	23.64
		Sd	1.17	0.29	0.82
	Female	X̄	59.88^{b}	11.92^{c}	23.46
		Sd	0.77	0.47	0.77
p-value					
Source of variation					
Protease			0.528	0.026	0.220
Sex			0.018	0.001	0.632
Protease x sex			0.827	0.676	0.415

X̄ - Average, Sd - Standard deviation
$^{a-c}$ Means within columns with different superscripts differ significantly (P<0.05)

Carcase characteristics assay - percent yields of individual meat classes (Table 3) demonstrated that different diet formulations used for the experimental groups of broilers had a considerably lower effect on the all class meat. Namely, that only dietary treatments had a significant effect (P<0.05) on the percentage yield of class II meat (control males had lower percent yields of class III meat compared to E-I male broilers), whereas that of class I and III meat was not significantly affected by this factor (P>0.05). The results in table 3. suggested that male birds had a significantly higher percent yields of class II meat (wings) compared to female birds (P<0.05). The percent yield of class I and III meat was not affected by sex (P>0.05).

Mohammadigheisar and Kim (2018) reported that carcase characteristics were not affected by the addition of exogenous protease to the diet of broiler chickens. Also, Dessimoni et al. (2019) determined that there were no effects on carcass and breast yield of male Cobb 500 in fattening that lasted 42 days when the contents of digestible amino acids were reduced (lysine, methionine, and threonine) with the addition of protease.

Bogosavljevic-Bošković et al. (2011) reported that no significant differences were observed in the percentage of class I, class II and class III meat between the sexes of broilers (P>0.05) for the Hybro broilers, while Young et al. (2001) states that there is no difference between percentage of wings - class II meat.

Conclusion

The results showed that the no significant differences were observed in the weight of class I and class II meat between dietary treatments (P>0.05), as well as that the reduction of crude protein by 4% in diets supplemented with 0.2% protease affected the weight class III meat in male chickens (P<0.05). The feeding treatments did not affect percentage of class I and class III (P>0.05), while significant differences were observed in percentage of class II meat (between male broilers in C and E-I groups, P<0.05). The effect of sex was significant on both weight and percentage of all three meat categories (P<0.05).

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APPLE POMACE UTILIZATION IN FATTENING PIGS DIET – EFFECT ON PRODUCTION PERFORMANCE AND CARCASS QUALITY

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Abstract

Apple pomace is a by-product of apple juice production and a rich source of carbohydrates, crude fibre, minerals and polyphenols. In line with the global tendency of reducing and reusing waste and food by-products, the aim of this research was to determine the effect of feeding diets containing dry apple pomace, on production performance and carcass quality of fattening pigs. The research included 40 crossbred pigs (Swedish Landrace x Large Yorkshire), assigned to 2 dietary treatments (20 animals per treatment). In the first dietary treatment – T1, pigs were fed conventional diets, containing 16% crude protein in the first fattening phase (25 – 60 kg body weight) and 13% crude protein in the second fattening phase (60 -100 kg body weight). In the second dietary treatment - T2, pigs were fed diets with the same protein content as in T1 treatment, supplemented with 7% and 10% dry apple pomace during the first and the second fattening phase, respectively. Standard pig fattening technology was applied. Results of the experiment have shown that apple pomace addition in fattening pigs' diets did not have negative effect on production performance and carcass quality, i.e. final body weight (T1: 100.10 *vs.* T2: 100.90 kg), average daily gain (T1: 0.585 *vs.* T2: 0.588 kg), feed conversion ratio (T1: 3.46 *vs.* T2: 3.48), dressing percentage (T1: 81.24 *vs.* T2: 80.83%), rump back fat thickness (12.50 *vs.* 12.95 mm) and meatiness (T1: 58.38 *vs.* T2: 57.69%). Based on the obtained results, it can be concluded that dry apple pomace can be used as a feedstuff in diets for pigs, as a substitute for other nutrients and thus contribute to environmental protection and conservation of natural resources.

Keywords: *apple pomace, by-product, fattening pigs, feed.*

Introduction

Meeting the growing demand for animal feed, in accordance with the principles of sustainable development, implies efficient utilization of existing resources. Therefore, special attention should be payed to the underutilized food industry by-products as potential feedstuffs (Bakshi *et al.*, 2016). Many food by-products are well known and already used in animal nutrition and feed production, such as oil meals, cereal bran, middlings, distillery by-products, etc. On the other hand, fruit and vegetable processing by products have to be well examined in order to become conventional feedstuffs. Apple pomace is a by-product of apple juice and cider processing industry and represents about 20–35% of the initial apple weight (Vendruscolo *et al.*, 2008; Suárez *et al.*, 2010). It is a solid residue, left after pressing of apples, which consists of mainly of apple skin/flesh (95%), seeds (2–4%) and stems (1%) (Bhushan *et al.*, 2008). Apple pomace is a rich source of complex disaccharides carbohydrates - cellulose, hemicellulose, lignin and pectin, simple carbohydrates - fructose and glucose, sucrose, polyphenolic compounds, minerals and contains less protein, fat and vitamins (Vendruscolo *et al.*, 2008; Sato *et al.*, 2010; Gabriel *et al.*, 2013). Several million metric tons of apple pomace are generated every year throughout the world (Kammerer *et al.*, 2014). Since apple pomace

is an organic material, with high moisture content (above 70%), rich in sugars, therefore easily microbiologically contaminated, its direct disposal to the soil in landfills causes environmental problems (Lyu *et al.*, 2020). In order to be used in animal nutrition over a longer period, apple pomace has to be preserved by ensiling or drying (Mirzaei - Aghsaghali *et al.*, 2011). Several studies have shown that fresh (wet), ensiled or dried apple pomace apple pomace can be successfully used as an energy feed for ruminants and can replace conventional feedstuffs, such as corn and other cereals (Ahn *et al.*, 2002; Ghoreishi *et al.*, 2007; Beigh *et al.*, 2015; Mirzaei - Aghsaghali *et al.*, 2011). Although a high fibre feed, apple pomace can be used to a limited extent in non-ruminants nutrition, such as broilers, laying hens, pigs, as a partial replacement for corn, due to its high sugar content (Zafar *et al.*, 2005; Pieszka *et al.*, 2017). Results of the research by Bowden & Berry (1959) showed that using up to 20% dry apple pomace in fattening pigs rations, did not have negative impact on production performance and carcass quality. Using apple pomace in pig nutrition may have health benefits, especially regarding the reduction of the negative effects of mycotoxins in feed. Sehm *et al.* (2007) reported positive effect on piglets' health when 3.5% apple pomace was added to the diet. Gutzwiller *et al.* (2007) found that the addition of dry apple pomace to feed mixtures contaminated with mycotoxins of the genus *Fusarium*, significantly reduced negative impact that such diet had on pigs' health and production results.

The objective of the present research was to investigate the effect of the apple pomace addition in rations of fattening pigs during the whole fattening period, and its effect on growth performance, feed efficiency and carcass quality.

Material and Methods

The feeding experiment was carried out in 2016. in the commercial pig farm within the company Kotlenik promet, Kraljevo (Serbia), with 40 crossbred pigs (F1 generation of Swedish Landrace x Large Yorkshire), 20 male and 20 female, with an average initial body weight of 27.22 kg. Pigs were assigned to 2 dietary treatments (T1 – control group i T2 – experimental group). Males and females were uniformly allocated by treatments in 2 separated pens (20 pigs/pen, 10 male and 10 female). The experiment lasted 125 days and it was divided in two phases, i.e. the first fattening phase (55 days) and the second fattening phase (70 days). In the first dietary treatment – T1, control group was fed conventional diets, i.e. concentrated feed mixtures, containing 16% crude protein in the first fattening phase (25 – 60 kg body weight) (diet T1F1) and 13% crude protein in the second fattening phase (60 -100 kg body weight) (diet T1F2). In the second dietary treatment - T2, pigs were fed diets with the same protein content as in T1 treatment, supplemented with 7% and 10% dry apple pomace during the first and the second fattening phase, respectively (diets T2F1 and T2F2).

Standard pig fattening technology was applied and *ad libitum* supply of both food and water was provided. Accommodation and feeding conditions were in line with the farm - industrial system and veterinary and sanitary interventions were done in accordance with the valid regulations of the Republic of Serbia. Dry apple pomace was obtained from the fruit processing factory Vino Župa, a.d., Aleksandrovac, after drying wet apple pomace, left after juice production. The chemical composition of dry apple pomace (moisture 7.92%, crude protein 5.90%, crude fat 2.26%, crude fiber 20.76% and total sugar 30.29%) was determined previously by Maslovarić *et al.* (2015), and was used to formulate the mixtures for the present research. All feed mixtures were formulated following the requirements of the National rulebook on the quality of the animal feed (Rulebook on the animal feed quality, 2014), by using feed formulation software Optimix, and corresponded to the age, category and nutritional requirements of fattening pigs (Table 1).

Table 1: The composition and the nutritive value of the diets

Diet composition	**Diets**			
	First fattening phase		**Second fattening phase**	
Ingredients (%)*	T1F1	T1F2	T2F1	T2F2
Barley	26.50	26.00	31.50	29.00
Corn	35.80	35.30	33.30	33.30
Soybean meal (44% crude protein)	6.00	6.00	-	-
Full fat soybean grits	6.00	6.00	7.00	7.50
Sunflower meal (32% crude protein)	7.00	7.00	7.00	7.00
Fodder yeast	1.50	2.50	2.00	4.00
Dry apple pomace	-	7.00	-	10.00
Wheat bran	14.00	7.00	16.00	6.00
Mineral and vitamin premix	3.00	3.00	3.00	3.00
Mycotoxin adsorbent	0.29	0.20	0.20	0.20
Calculated chemical composition (%)*				
Moisture	13.90	12.95	13.73	13.11
Crude protein	15.46	15.24	13.85	13.83
Crude fat	3.48	3.56	3.64	3.76
Crude fibre	5.48	6.43	5.43	6.75
Ash	3.47	3.33	3.36	3.18
Nitrogen free extract	50.98	54.01	59.99	59.37
Metabolisible energy, MJ/kg	12.52	12.58	12.42	12.49

*expressed on the fresh weight basis

Body weight was measured individually at the beginning of the experiment, at the end of the first and the second fattening phase (*i.e.* at the end of the trial). Individual average feed consumption was calculated based on the total quantity of feed consumed divided by the number of animals in the group. At the end of the experiment pigs were slaughtered at the Kotlenik promet abattoir. One-hour *post mortem*, the weight of carcass sides, rump back fat thickness and the thickness of *Musculus longissimus dorsi* were measured. Rump back fat thickness (S) (with skin) was measured with a ruler in the thinnest area, i.e. where *Musculus gluteus medius* grows into adipose tissue the most (mm). The thickness of *Musculus longissimus dorsi* (M) was measured as the shortest connection between the front (cranial) end of the *M. gluteus medius* and the upper (dorsal) edge of the vertebral canal (mm). Based on the measured values, dressing percentage and meatiness (muscle percentage - MP, %) were determined. Dressing percentage was calculated by dividing the hot carcass weight by the final body weight of the pigs. Meatiness (MP) was estimated following the Two-points method as MP=65.93356-0,17759*S+0.00579*M-52,54737*S/M, according to the Draft Rulebook on the quality of slaughtered pigs and the categorization of pork (2009).
Statistical analysis of the experimental results was performed by one-way ANOVA (analysis od variance) and the Tukey's HSD test at 95% confidence ($p<0.05$) (Statistica, 2013).

Results and Discussion

The results of dietary treatments on pigs' performance are presented in Table 2.
In the first fattening phase average daily gain (ADG) was almost the same for T1 and T2 group, while in the second fattening phase slightly higher ADG was recorded for T2 group, but this difference was statistically insignificant. Observing the whole fattening period, achieved values of ADG (0.585 *vs.* 0.588 kg) and the final body weight (100.10 *vs.* 100.90 kg) for T1 and T2 group were very similar, without statistically significant differences. Average daily feed intake (ADFI) in the first fattening phase was slightly lower for T2 group, while in the second fattening phase T2 group had higher ADFI as compared to T1 group. At

the level of the whole fattening period, differences between ADFI values were minor (2.02 *vs.* 2.05 kg) and statistically non-significant. Feed conversion ratio (FCR) was higher for T1 group in the first fattening phase, as opposed to the second fattening phase when higher FCR was recorded in T2 group. Considering the whole fattening period, similar FCR values were recorded for T1 (3.46) and T2 (3.48) group.

Table 2. Effect of dietary treatments on pigs' production performance

Trait	**T1**	**T2**
	$\bar{x}$, Sd	$\bar{x}$, Sd
Initial body weight, kg	27.05±1.28	27.4±1.19
Final body weight, kg	100.10±2.92	100.90±2.61
First fattening phase		
Average daily gain (ADG), kg	0.604±0.029	0.603±0.033
Average daily feed intake (ADFI), kg	1.85±0.20	1.78±0.27
Feed conversion ratio (FCR), kg feed/kg gain	3.06	2.95
Second fattening phase		
Average daily gain (ADG), kg	0.569±0.021	0.576±0.021
Average daily feed intake (ADFI), kg	2.15±0.22	2.26±0.24
Feed conversion ratio (FCR), kg feed/kg gain	3.78	3.91
Whole fattening period		
Average daily gain (ADG), kg	0.585±0.021	0.588±0.017
Average daily feed intake (ADFI), kg	2.02±0.21	2.05±0.23
Feed conversion ratio (FCR), kg feed/kg gain	3.46	3.48

Data are presented as the mean value ($\bar{x}$) ± standard deviation (Sd)

In the available literature limited data can be found regarding the effects dry apple pomace utilization in fattening pigs' nutrition. The results of the present research are in line with those obtained by Bowden & Berry (1958), who did not record statistically significant differences in daily gain, feed intake and feed conversion, when 10% of dry apple pomace was added to feed mixtures for fattening pigs. Also, Yamamoto *et al.* (2003) showed that the addition of 10% dry apple pomace to concentrated feed mixtures for fattening pigs weighing 80 to 105 kg, did not have a statistically significant effect on daily gain. On the other hand, in the study by Pieszka *et al.* (2017), addition of 8% dry apple pomace to feed mixtures for fattening pigs in the first fattening phase and 10% in the second fattening phase, led to a significant reduction in daily feed consumption, daily gain, and FCR. In order to achieve the same body weight, the fattening period lasted 4 days longer for the group fed diets with apple pomace.

The results of dietary treatments on pigs' carcass quality are presented in Table 3. Analysis of carcass quality parameters showed that no significant group differences occurred in warm carcass sides weight and dressing percentage, rump back fat thickness and carcass meatiness ($p>0.05$). Warm sides weight was slightly higher in the group fed apple pomace diet (T2), compared to the group fed standard diet (T1). On the other hand, dressing percentage and meatiness were somewhat higher in T1 group. Also, it was found that rump back fat thickness was slightly higher for T2 group.

Table 3. Effect of dietary treatments carcass quality of pigs

Parameter	**T1**	**T2**
	$\bar{x}$, Sd	$\bar{x}$, Sd
Weight of warm carcass sides, kg	81.35±2.06	81.55±2.03
Dressing percentage, %	81.24±1.08	80.83±1.26
Rump back fat thickness (S), mm	12.50±4.36	12.95±4.60
Thickness of *M. longissimus dorsi* (M), mm	70.70±6.00	69.30±7.13
Meatiness (MP), %	58.38±4.52	57.69±4.77

Data are presented as the mean value ($\bar{X}$) ± standard deviation (Sd)

Results of the carcass quality examination can be related to the results of the research by Yamamoto *et al.* (2003) in which no statistically significant differences in back fat thickness were recorded between the control group of fattening pigs (80 to 105 kg body weight) fed the standard feed mixture and the experimental group fed mixture supplemented with 10% dry apple pomace. Furthermore, Bowden & Berry (1958) found that the addition up to 20% dry apple pomace to feed mixtures did not have a significant effect on the carcass quality of fattening pigs. However, the in the study by Pieszka *et al.* (2017) the addition of 8 and 10% dry apple pomace to fattening pigs' diet, did not affect the weight of warm carcass sides, but led to a significant increase of the back fat thickness.

Conclusions

The results of the present research showed that addition of dry apple pomace to the diets for fattening pigs did not cause any significant differences in production performance and carcass quality. Therefore, it can be concluded dry apple pomace can be safely used as a feedstuff in fattening pigs' nutrition, i.e. 7% during first fattening phase (25-60 kg body weight) and 10% in the second fattening phase (60-10 kg body weight). The present data can be considered as preliminary since further research should be focused on more detailed examination of apple pomace share in the diets for different pig categories and production economy, as well as using dry apple pomace as a raw material in animal feed industry. Aforementioned would contribute to sustainable management of natural resources, reduction of organic waste and its negative impact on the environment.

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THE MERTOLENGA CATTLE BREED EXPLOITED IN THE MONTADO SYSTEM

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Abstract

Beef cattle production is an important component of sustainable agriculture in vast regions of Europe, ranging from maintaining the rural landscape to genetic preservation. Among the investigation topics covered are the various existing systems that arise as a result of the different uses of the same landscape. In Portugal, the "montado" system despite having a lot of bibliography dedicated to its ecology and landscape value, does not have much information gathered about the cattle holdings that use it. Such systems are characterized by the use of the herbaceous substrate under cork or holm oak, in strong integration with agricultural systems that produce cereal grains, straw and stubble, in addition to the production of hay and silage. In this perspective, the "montado" is a highlighted system, since its sustainability has been verified for centuries; however, with regard to its use by cattle, the information gathered is still insufficient and the economic efficiency of its farms is globally little known. The Mertolenga cattle breed withstand the most severe climatic conditions, being therefore explored in "montado" systems completely in the open, being one of the most representative breeds of this type of system. Being a small breed, it has lower energy maintenance needs, but with sufficient milk capacity to offer milk to the calves until weaning even when used in an industrial crossing. Another advantage over the larger and heavier breeds is the greater number of normal heads per hectare of grazing due to lower feed requirements.

Key words: *Cattle, Silvopastoral, Mediterranean, Pastures, Systems*.

Introduction

Beef cattle production is an important component of sustainable agriculture in vast regions of Europe, ranging from maintaining the rural landscape to genetic preservation, being one of the factors that contributed to the preservation of a high number of breeds in all countries of the European Union (Wolfová *et al.*, 2005).

However, despite the drop in global beef consumption, there was an increase in demand for meat from indigenous breeds, which, associated with extensive systems, namely in the cork oak region (*montado*), began to attract the attention of consumers, regardless of price. These differentiated products, despite representing only a small proportion of meat production and consumption in Portugal, have grown significantly (Viegas *et al.*, 2012).

Due to this trend, the analysis of the efficiency of beef cattle production systems in extensive regime has deserved investigation by several authors. Among the topics covered are the various existing systems that arise as a result of the different uses of the same landscape and consequent differences in the management of animals, in search of an optimal solution on the part of the breeder (Capillon, 1985; Liénard *et al.*, 1996).

In the case of Portugal, the *montado* environment occupies a prominent place, both for the area occupied by the system, and for the size of the explorations that characterize it, especially in Alentejo, a vast region in the southern Portugal. This system gradually replaced the original Mediterranean ecosystem, simplifying its biodiversity until it became the extensive silvo-pastoral system characteristic of the region. However, these characteristics

have undergone several transformations throughout the 20th century, resulting in soil degradation and contributing to the modification of the local climate (Ferreira, 2001).
In Spain, the *dehesa* system - equivalent to the Portuguese *montado* - despite having a lot of bibliography dedicated to its ecology and landscape value, does not have much information gathered about the cattle holdings that use it (Milán *et al.*, 2006). In terms of sustainability, the *montado* and the *dehesa* are reference systems since they have remained practically unchanged for centuries. However, their use by cattle has little information gathered and the economic efficiency of their farms is, overall, little known.
Knowing the extent to which the acorn production in the *montado* contributes to the dietary supplementation of cattle in extensive regime and how it influences the calving seasons of the Mertolenga breed, was the core of this study.

Material and Methods

The *montado* system is characterized by the use of the herbaceous substrate under cork oak or holm oak (the *montado* main species), in strong integration with the agricultural systems that produce cereal grains, straw and stubble, in addition to the production of hay and silage (Andrade *et al.*, 1999).
Among the characteristics of indigenous Mediterranean breeds, maternal aptitudes and high rusticity are particularly prominent in the Mertolenga breed (Andrade *et al.*, 1999; Beja-Pereira *et al.*, 2003; Roquete, 2004). According to the Breed's Genealogical Book, the Mertolenga breed may have roan or *thousand-flowers*, unicolor (red) and red spotted coat; animals with spotted heads or white handles are not allowed. It is a breed of nervous temperament and high rusticity, presenting small size, thin skeleton, clear or slightly pigmented mucous membranes. According to Felius (1995) *cit in* Costa, (2008) "the Mertolenga breed is very heterogeneous, probably descending from the Berrenda breed with modifications introduced by the Alentejana, Brava and Andaluza breeds".
In the specific environment of the Portuguese *montado*, the Mertolenga cattle is fully exploited in the open, being one of the most representative breeds of this type of system. The breeding herds can vary between 5 and 600 cows with an average distribution between 70 and 80. Depending on the region, environmental conditions, soils and grass cycles, there are well-marked breeding seasons (3 to 6 months) or, alternatively, the bull all year round in the herd.
Calves are weaned at around 6-8 months, whether or not they have been helped with concentrated food in the last 2-3 months, which helps the cow to recover some of its body composition, which is dependent on the calving season and the genotype of the calf.
Being a small breed, it has lower energy maintenance needs, but with sufficient milk capacity to offer milk to calves until weaning even when used in an industrial crossing with Charolais or Limousine (Rodrigues *et al.*, 1998; Mateus *et al.*, 2004; Roquete, 2004; Monteiro *et al.*, 2006). Another advantage over the larger and heavier breeds is the greater number of normal heads per hectare of grazing due to lower feed requirements.
Since upland pastures presents a great variation in productivity throughout the year, both in quantity and quality, the animals' food must be complemented by other resources such as straw and stubble during summer, in addition to hay and silage. In the *montado* the acorn production of holm oaks and cork oaks in the beginning of winter, works as a natural supplement that partially compensates for the low pasture production of this season (Cal, 2017).
In view of these dietary limitations, the reproductive management of the cow must be adapted to the pasture cycles and seasonal availability. As in Alentejo the most common reproductive system is matings throughout the year, the need for non-strategic supplementation due to the seasonal variation in food availability, leads to an increase in expenses (Reis, 2010). This

management is preferred by breeders because it is the simplest, but on the other hand it makes it impossible to coordinate health and reproductive management with food management, since the animals are at different stages of the production cycle.
Winter deliveries benefit from the quantity and quality of the first herbs at the end of winter, while cows that deliver in the summer have a poorer diet, consisting of the remaining spring pasture, cereal stubble and later on acorns, when on a *montado* pasture. The main disadvantages of each of these delivery periods are the poor conditions for the mother's milk production in the summer deliveries and the longer postpartum anestrus due to the negative effect of the photoperiod; in winter deliveries, the animals' body condition must be carefully observed and maintained, in order to not prolong the anestrus period even further (Leitão *et al.*, 2000; Lopes da Costa, 2008 *cit in* Cal, 2017; Reis, 2010).
According to Carvalho (1994), food availability throughout the year can be divided into four distinct periods:

1 - From october 1st to december 15th, when pasture production is reduced but its nutritional value is high. During this period, cattle must have, in addition to pasture, preserved foods such as straw, hay, silage and acorn in the *montado*; where necessary, it should also be provided some concentrated food.
2 - From december 16th to february 28th, pasture production is very low or even zero, since low temperatures prevent its growth. As in the previous period, the food quality of the grass remains high, but the reduced availability requires supplementation.
3 - From march 1st to june 30th, the conditions of temperature and humidity of the soil allow the rapid growth of the pasture, this being the period of greatest abundance of grass. However, in parallel with growth, there is a decrease in food quality, although this remains reasonable and allows the animals to feed exclusively on pasture, throughout this period.
4 - From july 1st to september 30th takes place the most critical period, due to the lack of water and the high temperatures. The animals feed on dry pasture and cereal stubble, and supplementation with hay and concentrates is often necessary.
When the intention is to privilege the milk capacity of the cow, the breeding season must be from april to june in order to deliveries take place from january to march. With this system, it is necessary to supplement the females during the phase of pregnancy that coincides with the time of lowest food availability (see Figure 1).

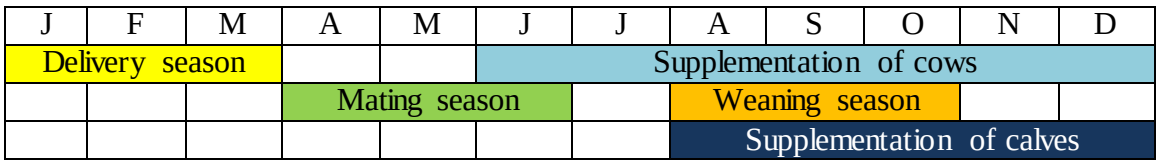


Figure 1 – Reproductive cycle model with winter delivery season (adapted from Lopes da Costa, 2011, *cit in* Cal, 2017)

If the breeder's option is to favor the growth of the calf after weaning, the calving season should be summer-autumn, so that the peak of pasture production in the spring coincides with the weaning season. In this system, the supplementation time for both cows and calves is substantially shorter, resulting in economic advantages for the breeder (see Figure 2). It should also be noted that, according to the proposed calendar, the end of artificial supplementation coincides with the beginning of the acorn production.

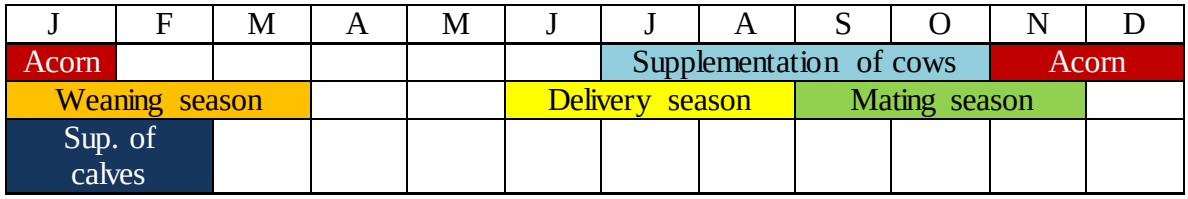


Figure 2 – Reproductive cycle model with summer delivery season (adapted from Lopes da Costa, 2011, *cit in* Cal, 2017)

The statistical analyzes that were carried out to evaluate the efficiency of the Mertolenga breed in various characteristics was as exhaustive as possible, having used the archives of the Association of Mertolenga Cattle Breeders (ACBM), which provided the required data (.TXT files) and subsequently transformed by us into .DFB files. The information collected provided a large amount of data for analysis, that was necessary to filter using own programs developed in CLIPPER language, and the Excel utility tools, in a way to extract the "noise" contained in the information.

The descriptive statistical analysis included the standard deviation, maximum and minimum values, mean and median; distribution asymmetry and kurtosis were also tested; normality was tested by the methods of Shapiro-Wilk, Anderson-Darling, Martinez-Iglewics, Kolmogorov-Smirnov, D'Agostino Skewness, D'Agostino Kurtosis and D'Agostino Omnibus, whenever it was understood to better understand the behavior of the analyzed characteristic. Subsequently, an analysis of variance based on GLM (Generalized Linear Model) processes was performed with a means comparison test, using the NCSS software package (Number Cruncher Statistical System, 2000 (Hintze, 1999))

Results and Discussion

In Alentejo, the climatic conditions observed in winter and summer require special attention for different reasons. In winter, rain, wind and low temperatures can affect productivity, especially that of younger animals; in summer, the high temperatures associated with the lack of water, strongly affect the availability and quality of food; if the breeder chooses to have the breeding season coinciding with the hot season, the fertility rate decreases, as bulls try less often to copulate and cows have progesterone levels negatively affected by high temperatures, which is reflected negatively on the estrous cycle (Pereira, 2006 *cit in* Cal., 2017).

In winter, postpartum anestrus are longer for cows that deliver at this season even though they have high nutritional levels, which suggests an effect of the photoperiod on reproduction (Leitão *et al*., 2000 *cit in* Costa, 2015).

The set of information collected for this work is not properly indicated for transforming the variables in order to obtain agreement with the normal distribution, but rather to analyze with the most original information possible. Therefore, more than manipulating information, the idea is to manipulate generalized linear models (GLM) taking into account the enormous amount of information.

Since the monthly distribution of deliveries allows a finer analysis of their distribution, an analysis of variance (GLM) was carried out to assess the extent to which the factors of variation had a significant effect on the parameters studied. Then, a Tukey-Kramer test was performed for the purpose of separating means.

Since the monthly distribution of deliveries allows for a finer analysis of their distribution, an analysis of variance was carried out to assess the extent to which the factors of variation had a significant effect on the parameters studied and thus allow for some statistically supported

influence that could clarify the efficiency associated with the characteristic in question. Table 1 shows the analysis of variance table corresponding to the GLM model "nested" used.

Table 1. Analysis of variance table relative to the percentage of monthly birth distribution

Source	Degrees of fredom	Sum square	Mean square	F-Test	Probability level (P)
A: CETP	3	2.487604	0.8292015	1.41	0.266232 NS
B(A): ANO	23	13.55757	0.5894595	0.00	1.000000 NS
C: MES	11	64591.31	5871.937	19.51	0.000000*
AC	33	69347.03	2101.425	6.98	0.000000*
BC(A)	253	76161.86	301.035	1.90	0.000000*
S	51553	8158821	158.2608		
Total (Adjusted)	51876	8438624			
Total	51877				

When applying the values in the "nested" model:

$Y_{ijkl} = \mu + CETP_i + ANO(CETP)_{i(j)} + MES_k + CETP*MES_l + ANO*MES(CETP)_{ik(j)} + e_{ijkl}$

Wherein:

Y_{ijkl} – i-th result of the parameter under analysis;

μ - average observed value of the study population;

$CETP_i$ – fixed effect of the i-th Stage;

$ANO(CETP)_{ij}$ – fixed effect of the year of delivery in the Stage j-th;

MES_k - fixed effect of the month of delivery k-th;

$CETP*MES_l$ - double interaction of the CAP Stage with the month of delivery;

$ANO*MES(CETP)_{ik(j)}$ - triple interaction of the year of birth with the month of delivery and CAP Stage;

e_{ijkl} – error.

it appears that between stages there were no significant differences, but throughout the year there are differences between months, with emphasis on the low number of births that occurred in June and July (see Table 2). When breeders choose this time of year, cows have long since weaned calves, usually before the end of winter. With this management, the peak of the grass that occurs in the spring is used to restore the body condition of the females, which allows the return to cyclicality; however, in the following months of calf suckling, only dry pastures and stubble abound, which requires adequate supplementation in quantity and quality, so that there is no excessive break in body condition. In the present case, it appears that this situation was avoided by the fact that births were concentrated in the two months that mark the end of winter and the beginning of spring (February and March - see Table 2), thus indicating that the breeders privileged the capacity of the females with a view to achieving greater weaning weights which requires a greater effort of supplementation (see Figure 1), despite the acorn production that takes place from November to January as seen in Figure 2.

It is therefore not to be excluded that breeders take market fluctuations into account when making decisions regarding reproductive management, preferring to shift the calving season on the calendar to the months when it would be easier for females to wean heavier calves.

Table 2. Percentage of deliveries observed per month and comparison of means

Month	Observations	Mean (%) ± Standard error	Tukey-Kramer
March	4326	10.012 ± 0.264	**a**
February	4328	10.010 ± 0.264	**a**
September	4320	9.810 ± 0.264	**ab**
January	4330	9.680 ± 0.264	**ab**
April	4326	9.294 ± 0.264	**ab**
December	4317	8.971 ± 0.264	**abc**
August	4322	8.709 ± 0.264	**bcd**
October	4314	7.818 ± 0.264	**cde**
May	4325	7.788 ± 0.264	**cde**
November	4317	7.178 ± 0.264	**ef**
July	4324	6.001 ± 0.264	**fg**
June	4328	4.953 ± 0.264	**g**
	Total : 51877		

It is also appreciable the low percentage of deliveries that occurred in the interval of 2 months after the month of calving, as a result of the generalized practice of having the bull permanently with the cows.

The analysis of variance carried out on the intercalving interval in relation to the breed phenotypes registered significant differences. Table 3 shows the analysis of variance table.

Table 3. Percentage of deliveries observed per month and comparison of means

Source	Degrees of fredom	Sum square	Mean square	F-Test	Probability level (P)
A: CETP	3	1.769641E+07	5898804	2.20	0.115140 NS
B(A): AP	23	6.161246E+07	2678803	134.32	0.000000*
C: CRAC	2	7960703	3980352	39.23	0.000000*
AC	6	5860881	976813.4	9.63	0.000001*
BC(A)	46	4667274	101462.5	5.09	0.000000*
D:NP	13	7.719442E+07	5938032	297.75	0.000000*
E:SV	1	16864.96	16864.96	0.85	0.357781 NS
F:MP	11	2.426527E+07	2205934	110.61	0.000000*
S	180318	3.596031E+09	19942.72		
Total (Adjusted)	180423	3.804389E+09			
Total	180424				

For the purposes of studying the phenotypes we use only the corresponding values, namely those referring to the CRAC code in the "nested" model:

$Y_{ijklmno} = \mu + CETP_i + AP(CETP)_{j(i)} + CRAC_k + CETP*CRAC_{ik} + AP*CRAC(CETP)_{jk(i)} + NP_l + Sx_m + MP_n + e_{ijklmno}$

Wherein:

$Y_{ijklmno}$ – i-th result of the parameter under analysis;

μ - average observed value of the study population;

$CETP_i$ – fixed effect of the CAP Stage i-th;

$AP(CETP)_{j(i)}$ – fixed effect of the year of delivery in the CAP Stage j (i) -th;

$CRAC_k$ – fixed effect of the k-th breed phenotype;

$CETP*CRAC_{ik}$ - double interaction of the CAP Stage with the breed phenotype;

$AP*CRAC(CETP)_{jk(i)}$ – triple interaction of the year of birth with the breed phenotype and CAP Stage;

NP_l - fixed effect of l-th delivery number;
Sx_m - fixed sex effect of the m-th calf;
MP_n – fixed effect of month of delivery n-th;
e_{ijklm} – error.

and obtained the following results that are presented in Table 4.

Table 4. Intercalving interval deliveries (in days) according to Mertolenga breed phenotypes

Phenotype	Observations	Mean (%) ± Standard error	Tukey-Kramer
Roan	100259	441.401 ± 1.006	**a**
Spotted	18857	462.787 ± 2.320	**b**
Red	61308	475.481 ± 1.286	**c**
	Total : 180424	**Global mean: 459.890**	

Conclusion

The fact that deliveries are concentrated in the two months that mark the end of winter and the beginning of spring (february and march), reveals that in general the breeders favored the milk capacity of the cows, by providing that they benefit from the peak of grass production that occurs from march on, with the aim of increasing the weights of calves at weaning. On the other hand, the final stage of pregnancy partially coincided with the acorn production that works as a natural complement provided by the *montado* system, which contributes to mitigate the lack of pasture that is felt in the beginning of winter, although it is not enough in the most cases. Contrary to this practice, in the region to the east of the Guadiana river, summer deliveries (in august and september) were always chosen, so that the production of spring grass would benefit the female's body condition before calving. As for the phenotypes, the roan presents the best results for the intercalving interval and the spotted, the worst; however, attention should be drawn to the fact that roans are exploited in denser *montado* regions, while the spotted herds are geographically more concentrated in the region to the east of the Guadiana river, where the *montado* is less dense and the climatic conditions less favorable, due to less precipitation, which may contribute to the worst results of this phenotype. On other hand, the percentage of deliveries that occurred in two consecutive months remains low because a large number of farms persist in the simplistic model of keeping the bull permanently with the cows.

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HOW MUCH MEAT DO WE ACTUALLY EAT? AN ESTIMATION OF THE LOSS-ADJUSTED CONSUMPTION OF MEAT IN GERMANY

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Abstract

Because of various negative effects on the environment, health and animal welfare, meat consumption is on everyone's lips. Despite this, the statistical basis for the level of "consumption on the fork" or rather the level of loss-adjusted meat consumption, is subject to uncertainties. This is also true for Germany as the statistical method for calculating loss-adjusted meat consumption using fixed coefficients is based on estimations that have not been updated since they were first applied in 1989. Evidence from a representative national household survey indicated a per capita meat consumption of 42kg for Germany in 2007. The application of the coefficient, on the other hand, leads to a consumption level of 60kg, which has been at this level for years. The discrepancy of the two results indicates an overestimation of meat consumption using a 30-year old coefficient. Against this background, it is the objective of this study to estimate the loss-adjusted meat consumption for pork, beef, and poultry meat. The estimation is based on a material-flow-analysis including production data, distribution channels and losses along the value chain at seven different stages. Initial results show a slight reduction of the consumption level for pork of 6.16% compared to the old calculation, whereas the consumption level for beef and poultry has slightly increased by 16.16% and 14.49%.

Keywords: *Loss-adjusted meat consumption, material-flow-analysis, international comparability*

Introduction

Due to negative external effects, the consumption of animal proteins is currently under intense discussion in society and science. In particular, demand for meat is at the centre of social controversy, as an increased level of meat consumption is associated with several negative effects. Besides unfavourable impacts on human health, adverse effects on environment, climate, resources conservation and animal-welfare are observed (Bonnett *et al.*, 2020). Nevertheless, global demand for meat is increasing. Against this background, researchers at various national or institutional levels carry out impact assessments in order to change current demand behaviour and often recommend a reduction in meat consumption (Arneth *et al.*, 2019; Spiller *et. al.*, 2015; Isermeyer, 2019).

However, Willett *et al.* (2019) emphasise that the regulation of meat consumption will be one of the greatest challenges that industrialised countries will face in the coming years. In this regard, the assessment of any agricultural production policy would require a solid database, also regarding meat consumption. However, the consumption level shown in official national statistics represents a calculated quantity resulting from the net production adjusted by export and import. Thus, the calculated consumption level may differ from the actual amount of meat eaten by the consumer, as "consumption" includes non-edible parts (e.g. bones), non-food uses (e.g. material for pet-food production), losses and waste along the value chain. To solve

this problem a coefficient is used in Germany to deduct these fractions and estimate a loss-adjusted meat consumption level.
This coefficient, however, is based on assumptions that go back more than 30 years. It was first applied in 1989 and has hardly been adjusted ever since (Hühne, 1986). Doubts as to the realism of this approach are already apparent looking at the results of the National Nutrition Survey II (NVS II). The representative consumer survey reported a meat consumption level of 42kg per capita in 2007, whereas according to the supply balance, the loss-adjusted consumption level was between 60-62kg during the last 10 years (BLE, 2020; Heuer *et al.*, 2015). The discrepancy between these results points to a possible overestimation of consumption based on official statistics that can be attributed to a number of factors, such as an incorrect estimation of losses and waste along the value chain and non-food use. A general change in the way the market operates in terms of distribution or marketing channels for meat and meat products might also lead to an incorrect estimation.
Therefore, this paper proposes an updated estimation for the loss-adjusted meat consumption level for beef, pork, and poultry meat in Germany. The authors carry out a material-flow-analysis and include data on production, distribution channels and losses along the value chain at seven different stages. The loss-adjusted meat consumption level for pork, beef and poultry per capita in Germany is identified as well as strengths and weaknesses of the approach. The authors further discuss the international comparability of the findings.

Material and methods

Central to the estimation is the analysis of production data collected at the slaughterhouse level. The authors identified the production share available and edible for human consumption in Germany. The authors also differentiated between production share passed on to different food distribution channels (food retail, meat products industry and wholesale) measured in carcass weight equivalent. In addition, bones included were determined, as they are not edible and, therefore, have to be deducted from the amount available for human consumption. Exports as well as non-food uses (for instance the production of pet food, or the use in the pharmaceutical industry) are also recorded in the course of a plausibility check but are not included in the subsequent material-flow-analysis, as they are not available for human consumption in Germany.
The determined meat quantity still does not fully reach the consumer's fork, as there are losses in the process from slaughter to retail and waste at the consumer level that have to be calculated and deducted. Therefore, losses at the subsequent stages from slaughter to the consumer are calculated, further reducing the available amount of meat for human consumption. In this context, the authors take into account losses at food retail, processing-industry and wholesales and in addition waste at the household level and losses regarding consumption out-of-home. The model used for the calculation of loss-adjusted meat consumption is shown in Figure 1.

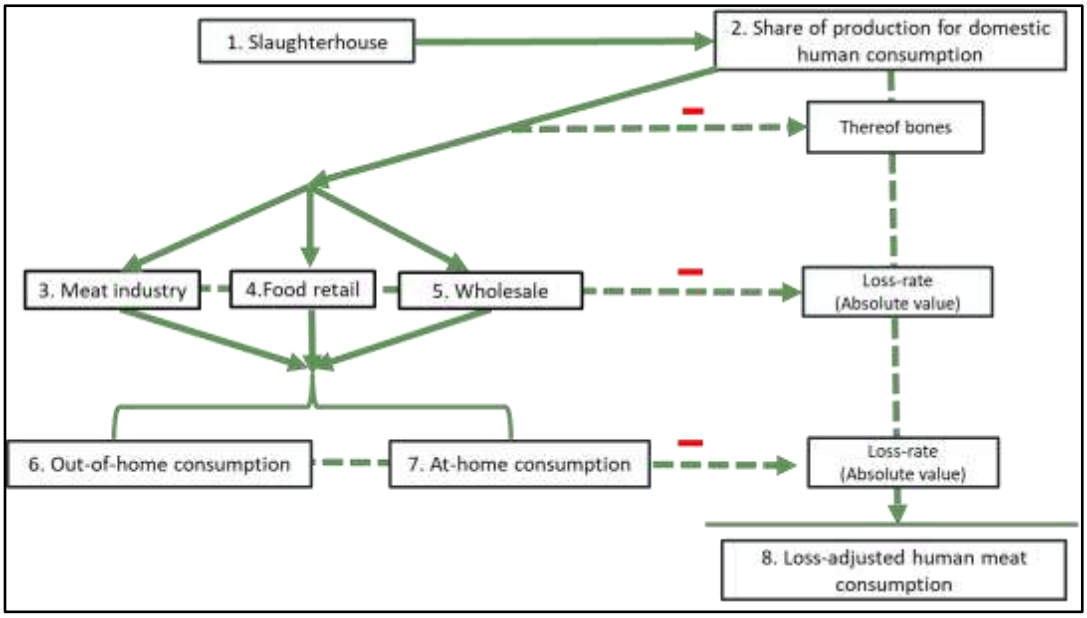


Figure 11. Calculation model for loss-adjusted human meat consumption.
*Source: Author's elaboration.

Data at slaughter level was collected using spreadsheets in Microsoft Excel. Together with experts from the industry, the authors developed a query mask to achieve a correct recording according to the slaughter process. In this way, the slaughterhouses were able to provide data coming from their Enterprise Resource Planning Systems (ERP). The authors might therefore be able to repeat the survey in the future.
The data was collected from October 2018 until May 2020. 2017 and 2018 are the reference years. The data also includes information regarding different marketing channels and utilization ways for individual products or product groups for the animal species pig, beef and veal, chicken and turkey. In terms of slaughter volume, the market coverage is 51% for pork, 51% for beef and 81% for poultry.
The query list is structured according to the slaughter process. Animal species-specific by-products from slaughter (edible in principle) and animal by-products (classified in risk categories 1-3 for further processing) needed to be reported first, as they are removed first from the carcass in the process of harvesting meat, followed by the total carcass weight and losses for cooling. Subsequently, meat utilisation ways were recorded. To do this, the authors divided the food channel into food retail, meat production industry (processing) wholesale and others. In addition, the individual bone proportions related to the specified products or product groups were queried. Loss-rates and waste-rates at the subsequence stages of the value chain were identified based on literature, expert interviews and a separate survey of the out-of-home market. Table 1 presents an overview of the individual loss-rates.

Table 1. Loss-rates measured in percentage (%)

Loss position	Percentage loss (%)	Source
Food retail	2.58	Lebersorger *et al*., 2014
Meat industry	0.1	Expert interviews
Wholesale	1.0	Expert interviews
At-home consumption	3.08	Hübsch *et al*., 2017
Out-of-home consumption	6.15	Own survey and calculations

The total loss-adjusted meat consumption level (without bones) is calculated by subtracting the shown loss rates without bones at the slaughter level. The projection of a loss-adjusted

meat consumption per capita for Germany is based on the net production (which is equal to the slaughter quantity) adjusted for exports and imports. This key figure can be taken from the official statistics in the supply balance sheet for meat. The total loss-adjusted meat consumption level is now divided by the number of inhabitants, in order to receive per capita figures.

Results and discussion

Using the official meat statistics, the loss-adjusted meat consumption level can be calculated and reported per capita as described above. The results for the different meat categories are presented in Table 2. The authors distinguish between pork, beef and poultry. The overall result for poultry is derived from the partial results for chicken and turkey. In addition, the percentage change in the level of consumption compared with the old estimation method is presented. From the results, it becomes clear that there was no apparent overestimation, but rather an underestimation of the beef and poultry consumption level.
Strikingly, results show a per capita consumption for beef of 11.51kg using the new estimation method, while the old method gives a per capita consumption of 9.94kg, representing a percentage change of 15.79%. The per capita meat consumption level for poultry increased by nearly 2kg. In contrast, the loss-adjusted consumption level for pork decreased by 6.38%.

Table 2. The estimated loss-adjusted consumption of beef, pork and poultry in kg per capita

Type of meat	Loss-adjusted human meat consumption in kg per capita; old estimation	Loss-adjusted human meat consumption kg per capita; new estimation	Percentage change (%)
Beef	9.94	11.51	+ 15.97
Poultry	13.80	15.78	+ 14.34
Pork	35.89	33.60	- 6.38

*Source: Author's elaboration.

Based on these results, the authors cannot confirm the initially formulated hypothesis of an overestimation of meat consumption using the old coefficients. The different methodological approaches of the estimations make the comparability of the results difficult. In the estimation carried out by Hühne et al. (1989) loss-rates were set mainly based on experts' assumptions. This is because analyses based on suitable data sources were not available. Second, contrary to our approach, losses at the slaughterhouse level and along the chain were deducted on a flat-rate basis, implying that the actual losses were not calculated based on quantities utilized at each stage of the value chain.
Due to the improved accuracy as well as the type of data used, the authors assume that the estimation method shown certainly leads to more detailed results. However, there are possible sources for inaccuracies. A fundamental challenge in this context was the comparison of data of different origins. While the carcass weight is measured at a certain point in the production process, information regarding marketing channels is based on sales data. Therefore, inconsistencies may arise in connection with the mixing of both data sources, as cuts might have been partially processed (freezing, marinating, etc.). Although these changes in weight were taken into account, they can still lead to inaccuracies. In addition, the internal definition of the carcass weight in slaughterhouses was not always consistent with the official statistics, as these are based on the marketing of the cuts. The proportions of bones included in certain cuts and products, however, are not weighted at any stage of the slaughter process. This is why employees of the slaughterhouses estimated them. The authors checked the information for plausibility.

The production share exported or used for food production and non-food production varies considerably from company to company but is decisive for the consumption level that we report. A larger sample would have been helpful in this context. However, as the companies expressed concerns about the workload and the sensitivity of the data, only a part of the companies contacted actually participated in the study. Thus, the level of consumption is not only linked to the sampling, but also the time of data collection. Since marketing structures can change depending on the market situation, the quantities marketed in the individual channels and thus the resulting losses may well be different in the future. In addition, the international comparison of meat on the fork is in general quite challenging because of different calculation or estimation methods. For example, Italy uses an approach based on a material-flow-analysis and loss-rates, which is similar to the one presented in this paper, but does not differentiate between at-home consumption and out-of-home consumption. Sweden, on the other hand, uses results of consumer surveys in order to recalculate the actual amount eaten by the consumer in "raw weight" (before cooking) (Russo *et al.*, 2016, Öberg, 2017).
These examples already show that the assessment methods used are likely to affect the findings, particularly at the international level. As marketing channels and loss-rates might be different in other countries, our findings cannot be generalized to the international level. This is why, the loss-adjusted consumption level might not be a reliable benchmark when implementing policies and analysing long-term trends. Hence, it is not surprising, that official statistics often show the level of consumption [net production deducted by exports and imports] (European Commission, 2020). In the course of public communication, however, the authors would like to emphasize that it is not human meat consumption that is compared here, but ultimately the available quantity, which still includes losses and waste, non-edible material and ways of utilization other than food production.

Conclusion

Against the background of negative external effects of demand behaviour, well-founded statements on the level of human meat consumption would be particularly useful for the implementation of political measures. Assessing human meat consumption from the supply side seems a practical solution. This paper presents an updated version of the estimation of loss-adjusted meat consumption for Germany. Compared to results from 1989 we found slightly increased consumption levels for beef and poultry, but a decreased level for pork. Nevertheless, methodological difficulties and information gaps regarding meat quantities and losses along the value chain possibly limit the findings of the study. As the marketing structures [exports, food production, or non-food production] of the slaughterhouses appeared to be diverse and were included in the analysis, the sampling is reflected in the results. This aspect also means that our findings may not be applicable at the international level. Different methodological approaches at the international level, in general, lead to the conclusion that consumption is not suitable as a reference level for international comparison. Therefore, it would currently be advisable to use net production adjusted by export and import for further analysis on an international level. Nevertheless, given the intensity of the discussion on negative consequences of human meat consumption, it is important to look for other approaches that will allow for international comparison.

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INFLUENCE OF SWEET CHESTNUT HYDROLYSABLE TANNINS ON SKATOLE CONCENTRATION IN *COLON DESCENDENS* AS IMPORTANT COMPONENT FOR APPEARANCE OF BOAR TAINT IN BOARS

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Abstract

Factors related to nutrition, especially certain diet ingredients, were shown to affect the compounds responsible for boar taint, namely skatole. Skatole is one of the two main compounds of boar taint. It is produced in the large intestine by bacterial degradation of amino acid L-tryptophan and after absorption, it is transported to the liver, where it is metabolized mainly by cytochrome P450 (CYP450) enzymes. Tannins are water-soluble polyphenols with varying molecular weights. They are chemically classified as hydrolysable or condensed tannins and are both considered to have either adverse or beneficial effects depending on their concentration and nature.

The aim of the paper is to present the effect of different levels of inclusion (up to 3%) of sweet chestnut hydrolysable tannins on chemical traits, skatole and indole concentration of intestinal content of *colon descendens* (CD) of entire males (EMs). Authors reported that different supplement of hydrolysable tannins reduce skatole and indole concentration in large intestine, especially in CD. Trials were carried out on crossbred (Large White × Landrace) and Swiss Large using mixed feed for growing and finishing period of raising with different inclusion of hydrolysable tannins (HTs) from 1-3%. Results showed constant reduction of intestinal skatole concentration in boars, from control to 3% group. Significant effect of tannin supplementation was also observed on nitrogen content in DM; as it was significantly higher with 2% and 3% than control group. Similar trend, although not significant, was reported for nitrogen content of wet matter. Ammonia content was reduced with tannin supplementation, but the difference was significant only between control and 1% supplemented group. No effect of tannin supplementation was observed on pH and indole content. Studies also reported that EMs supplemented with 3% of HTs positively affect proportion in intestinal bacterial flora of *Oscillospira* genus, *Ruminococcaceae* family, reduce amounts of *Lactobacillales* order, *Streptococcaceae* and *Veillonellaceae* families in the microbial ecosystem. The present findings suggest positive effects of tannins to decrease skatol concentration in intestinal content, as important component for appearance of boar taint in boars.

Keywords: *skatole, indole, large intestine, hydrolysable tannins, boars.*

Introduction

Boar taint is an unpleasant odour, often perceived when thermically cooking and eating meat from sexually mature male pigs. Two main compounds responsible for its presence are androstenone and skatole. Skatole, exhibiting a faecal-like odour, is produced by bacterial degradation of amino acid l-tryptophan in the hind gut and has no physiological function (Walstra and Maarse, 1970). Several factors, such as the formation, absorption, metabolism, and deposition influence the concentration of skatole in adipose tissue, which is largely under the influence of nutritional factors (review in Wesoly and Weiler, 2012; Bilić-Šobot et al., 2014).

Biosynthesis of skatole occurs in a two-step procedure, L-tryptophan is first converted to 3-indoleacetic acid, which is subsequently converted to skatole (for review see Jensen and Jensen 1998). Intestinal bacteria's *Escherichia coli* and *Clostridium spp*. are responsible for the production of indol-acetic acid, while indole-3-acetic acid is converted to skatole by *Lactobacillus sp*. (Honeyfield and Carlson, 1990; Deslandes et al., 2001). Important role for skatole concentrations in fat, have nutritional factors, by changing intestinal contents or the rate of passage and absorption (Jensen, 2006).

Tannins are water soluble polyphenols with varying molecular weights. They are chemically classified in two classes: hydrolysable and condensed tannins, and both are considered to have adverse and beneficial effects depending on their concentration and nature in animal feed (Bhat et al., 1998). In many animal species adding tannins in the diet had negative effects including hepatotoxicity, toxic nephrosis, feed intake depression and growth reduction as they reduce digestibility of proteins, lower the activity of digestive enzymes, cause damage to intestinal mucosa or exert systemic toxic effects (Mueller-Harvey, 2006). Also tannins have positive health effects, enhancing properties, such antibacterial, anti-parasitic, antioxidant, antidiarrheal and anticancerogenic in animals (Min and Hart 2003; Geidarn et al., 2007; Frankič and Solobir, 2011). Recently was investigated effects of hydrolysable tannins on histological and immunohistological properties in intestinal tract (Bilić-Šobot et al., 2016a), intestinal microbiota and influence on skatol and androstenone concentration in adipose tissue (Brus et al., 2013; Čandek-Potokat et al., 2015), growth performance (Brus et al., 2013; Bilić-Šobot et al., 2016b) in pigs and entire mails.

Tannins are known to have bitter or astringent taste which reduces palatability and consequently negatively affect pigs voluntary feed intake and growth performance. It has also been shown that they inhibit specific gastric enzymes (Murakami et al., 1992), lower proteolytic enzymes activity in small intestine (van Leeuwen et al., 1995). They decrease activity of trypsin in pancreatic tissue (catalyzes the hydrolysis of peptide bonds in duodenum) and activate brush border (γ-glutamyl transferase) which could disturb absorption of small intestine and pancreas in pigs (Lizardo et al., 1995; Bilić-Šobot et al., 2016a), reduce protein digestibility (Smulikowska et al., 2001) and increase the excretion of endogenous proteins (Jansman et al., 1995). On the other hand it has been shown that feeding weaned piglets with tannin wood extract can result in improved feed efficiency and reduction of intestinal bacterial proteolytic reaction (Biagi et al., 2010; Tretola et al., 2019), resulting in reduced count of harmful and elevated count of beneficial microorganisms in feces and increased growth performance during pre-fattening and fattening period (Brus et al., 2013). Recently published studies (Bilić-Šobot et al., 2016c; Tretola et al., 2019) observed no negative effects on growth performance in boars feed supplemented with hydrolysable tannins.

The aim of present study is to evaluate reported data of influence of hydrolysable tannins on skatole and indole concentration in intestinal content of *colon descendens*.

Skatole

Skatole is formed from amino acid L-tryptophan in large intestine of pigs and its biosynthesis occurs in a two-step procedure, L-tryptophan is first converted to 3-indoleacetic acid, which is subsequently converted to skatole (for review see Jensen and Jensen 1998).

Two major bacteria: *Escherichia coli* and *Clostridium spp*. are responsible for the production of indol-acetic acid, while indole-3-acetic acid is converted to skatole by *Lactobacillus sp*. (Yokoyama et al., 1977, Honeyfieled and Carlson 1990, Deslandes et al., 2001).

This implies that the production of skatole is primarily dependent on the availability of tryptophan and activity of intestinal bacteria. In view of the fact that pigs are non-ruminants they can tolerate high amounts of skatole, without affecting their health. Skatole is absorbed

from the intestines into the bloodstream and through V. Porte transferred to the liver (Jensen, 2006). The absorption of skatole is very fast, half-life in blood is 1h (Agergaard and Laue, 1993) and 11h in muscle and adipose tissue (Agergaard and Laue, 1994). In the liver occurs metabolism of skatole in two phases together with hepatic enzymes (for detailed explanations see reviews Wesoly and Weiler, 1999 and Bilić-Šobot et al., 2014). The enzymes CYP2E1 and CYP2A, isolated from cytochrome P450, are involved in this phase (Doran et al., 2002).
After that step, the non-degraded part is accumulated in adipose tissue (Squires and Lundstrøm, 1997, Babol et al., 1998, Zamaratskaia and Squires, 2008), and only a small proportion is excreted in the urine. Based on these findings, it is clear that hepatic metabolism plays an essential role in accumulation of skatole in fat (Zamaratskaia and Squires, 2008). The liver enzymes CYP2E1 and CYP2A are responsible for the production of skatole, but it can also be found in other tissues such as kidneys and lungs (Squires and Lundstrøm, 2011). In boars, low enzymes activity of both CYP2E1 and CYP2A is associated with high concentration of skatole in fat (Squires and Lundstrøm, 1997; Zamaratskaia et al., 2005).

Tannins

Chemically, tannins are not well-defined substances, but rather group of substances with the ability to bind proteins in aqueous solution (Makkar, 2003). Tannins as a supplement in animal diet have some positive and negative effects. They are tentatively classified into two classes: hydrolysable and condensed tannins and are considered to have both adverse and beneficial effects depending on their concentration and nature in animal diet (Mueller-Harvey, 2006).
Hydrolysable tannins are composed of estres of gallic acid or ellagic acids with a sugar core which is usually glucose, and are readily hydrolysed by acids or enzymes into monomeric product (Bhat et al., 1998). Commercial hydrolysable tannins are extracted from Chinese gall (Rhus semialata), sumac (Rhus coriata), Turchis gall (Quercus infectoria), tara (Caesalpina spinosa), myrobalan nuts (Terminalia chebula) and chestnut (Castanea sativa) review in Mueller-Harvey and McAllan (1992). In the past, tannins were often described as antinutritional factors because they can negatively impact animal production (Kumar and Singh, 1984; Lowry et al., 1996; Makkar, 2003). The response of ruminates and non-ruminates animals on tannins are lower feed intake, protein and dry matter digestibility, lower live weight gains, milk yield and wool growth (Jansman, 1993; Reed, 1995; Ramaha et al., 2019). In non-ruminants animals tannin can reduce the digestibility of proteins, increase the excretion of proteins and essential amino-acids, might lower the activity of digestive enzyme, may cause damage of mucosa of digestive tract or exert systemic toxic effects (Jansman, 1993). However, there is well-known practice of the use of tannin-rich feed in Iberian pigs from Mediterranean region. They have relish acorns and have traditionally been fed extensively during the fattening period on acorns from several oak species (Bruno-Soares and Abreu, 2003). Like results of these, meat often becomes marbled, and products have very high price (Cantos et al., 2003). These can be explained with fact that tannins and their derivates appear to animal species, as the first evolutionary defence mechanism against the protein precipitating activity of tannins, preferably bound to proline and histatins in saliva (Mehansho et al., 1983; Wróblewski et al., 2001; Cappai et al., 2010). Also are reported studies of influences of tannins like feedstuff in growing pigs on parotid gland (Cappai et al., 2010). Prevolnik et al. (2012) reported there was no influence on growth rate, carcass traits and meat quality with 0.2% of tannins in pigs' diet, Brus et al. (2013a) reported that supplementation with 0.2% of tannins in pigs diet have higher growth performance and lower necrosis which is associated with some changes in small intestinal morphology. In another study of Brus et al. (2013a) the chestnut tannin and organic acids as a supplement in pigs feed reduce the pathogens in faeces and improving the growth performance after weaning.

Results and Discussion

Table 1. The effect of supplementing the diet with tannin-rich extract (Farmatan) on final live weight

Concetration of tannins %	Initial live weight, kg	*p* value	Final live weight, kg	*p* value	References
1	51.7	0.99	124.7	0.33	Čandek-Potokar et al., 2015 first study
2	52.4		125.6		
3	51.7		115.5		
1	52.5	0.01	125.3	0.00	Bilić-Šobot et al., 2016 second study
2	49.7		129.9		
3	53.7		133.2		
3*+	65.8	0.54	108.5	0.76	Tretola et al., 2019 third study
3*-	66.8		110.4		

3*+: high polyunsaturated fatty acid (PUFA) with 3% chestnut extract containing HTs; 3*-: low PUFA with 3% chestnut extract containing HTs

Studies reported by authors Čandek-Potokar et al., 2015; Bilić-Šobot et al., 2016 and Tretola et al., 2019 indicate altered results on finale live weight supplemented with HTs diet. In the presented second study high concentration of Farmatan supplementation significantly increased body weight in 1%, 2% and 3% supplemented groups ($P < 0.01$). This results are not in agreement with results Čandek-Potokar et al. (2015), where live weight was significantly reduced in 3% supplemented group with Farmatan, likewise in the study of Tretola et al. (2019), final body weight were the same in all treatment groups. Also published data in the study Štukelj et al. (2010), indicate that 15% of Farmatan addition in growing pigs did not improve growth performance, but also was not violated.

Table 2. The effect of diet supplemented with tannin-rich extract (Farmatan) on pH, dry matter, indole and skatole concentrations in intestinal content of *colon descendens*

Concetration of tannins %	pH	*P* value	DM content, %	*P* value	Indole content, µg/g	*P* value	Indole in DM, µg/g	*P* value	Skatole content, µg/g	*P* value	Skatole in DM, µg/g	*P* value	N content, mg/g	*P* value	N in DM, mg/g	*P* value	NH_3 content, mg/g	*P* value	NH_3 in DM, mg/g	*P* value	References
1	7.31	0.14	22.6	0.22	3.52	0.73	15.8	0.14	8.46	0.00	37.11	0.00	7.95	0.33	35.95	0.00	0.33	0.02	1.47	0.78	Bilić-Šobot et al., 2016
2	7.28		22.0		3.16		15.2		7.97		34.76		8.70		44.56		0.36		1.83		
3	7.39		20.04		3.50		18.1		4.75		23.58		8.64		42.67		0.35		1.78		
1	7.27	0.22	25.2	0.24	/	/	19.4	0.19	/	/	64.5	0.01	/	/	41.7	0.00	/	/	2.23	0.11	Čandek-Potokar et al., 2015
2	7.17		26.5		/	/	14.4		/	/	62.5		/	/	43.3		/	/	1.41		
3	7.41		25.1		/	/	23.2		/	/	97.6		/	/	47.5		/		2.00		

*DM – dry matter.

In the experiment by Bilić-Šobot et al., 2016a and Čandek-Potokar et al., 2015 skatole concentration in *colon descendens* was significantly decreased in 3% treatment group then other supplemented groups ($P < 0.00$ and 0,01). Likewise, skatole concentration in dry matter was also significantly decreased in 3% supplemented group in comprising with other

treatment groups. ($P < 0.000$). Nitrogen concentration in form of dry matter was followed with increased concentrations between control and others groups ($P = 0.00$ and 0.00). Additional parameters pH, dry matter, nitrogen, ammonia in dry matter, indole and indole in dry matter were not significantly different between treatment groups (Table 2).

The ammonia concentrations (NH3) in the intestinal content was accompanied with considerable differences between control and 1% supplemented group and between 2% and 3% treatment groups ($P = 0.014$).

Recently published study (Tretola et al., 2019) investigate the effects of HTs tannins on gut microbial structure, *Lactobacillales*, *Streptococcus* and *Proteobacteria* bacteria compared to the group with no dietary chestnut supply, was reduced. This can explains reduced concentration of skatole in *descending colon*, as disrobed before, *Lactobacillales* are responsible for converting indole-3-acetic acid to skatole. This results can be associated with data published by Bilić-Šobote et al., (2016) where apoptotic cell count were significantly reduced in CD with diet supply with HTs.

Skatole originates from cell debris of the gut mucosa and it is product of intestinal bacterial metabolism of amino acid L-tryptophan (Jensen and Jensen, 1998). There are different ways to influence skatole levels, either by altering the microbial nitrogen metabolism or by changing the intestinal content the rate of passage and absorption (Jensen, 2006). Ammonia in gut intestinal content is purgative product produced from bacteria *E. coli* (which plays important role for skatole production) and in current study nitrogen in *c. descendens* is accompanied by decreased ammonia concentration (Table 2). This can be explained with fact that nitrogen has positive response with tannin supplementation, in feedback to the protein-binding property of tannins (Mueller-Harvey, 2006). Literature data on a variety of diet supplements to reduce skatole level in blood, adipose tissue and faces, in pigs are comprehensive (see reviews of Wesoly and Weiler 2012; Bilić-Šobot et al., 2014). In agreement with published data (Čandek-Potokar et al. 2015; Bilić-Šobote et al., 2016a; Bilić-Šobot et al. 2016b; Tretola et al., 2019), we observed a clear effect of tannin supplementation on (reduced) skatole production in the intestine, and results of decreased mitotic and apoptotic activity in the large intestine which may explain the decreased formation of skatole in the hindgut which indicates a beneficial effect on gut micloflora.

Conclusion

The presented results indicate positive effects of tannins to decrease skatol concentration in intestinal content, as one of important component of appearance of boar taint in boars.

In particular, hydrolysable tannins might exert potential for boar taint prevention via reduced intestinal skatole production.

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DETECTION OF *SALMONELLA* INFECTION IN BROWN RAT (*RATTUS NORVEGICUS*) ON PHEASANT FARM

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Abstract

Bacteria belonging to the genus *Salmonella* are significant cause of food poisoning in humans, and may also cause serious disease in poultry, and thus in pheasants. The most important serovars for the health of pheasants belong to the species *S. enterica*. One of the reasons for the presence of *Salmonella* on pheasant farms is the presence of infected rodents, primarily brown rats (*Rattus norvegicus*) in the holding yards and in the interior of farm buildings. The brown rat through secretions and excretions excretes *Salmonella*, which usually peros enters the digestive tract of pheasants and begins infection. In order to assess the risks of the presence of brown rats in a pheasant farm from the point of introduction of *Salmonella* in farm, we organized this experiment with the aim of capturing and detection of the presence of *Salmonella* in brown rats. The experiment was organized in the pheasant farm in April and May using specialized rat traps. During the experiment 35 specimens of brown rat were trapped. Control of specimens for the presence of *Salmonella* ascertained the presence of *S. enterica subsp. enterica serovar Enteritidis* in 7 specimens (20.0%).

Keywords: *Salmonella, infection, Rattus norvegicus, pheasant, pheasant farm.*

Introduction

Pheasant farms are semi-closed production facilities with several production units that are cyclically linked, which are bred different age groups of pheasants. Production units are aviary for the parent flock, a room for storing eggs, a hatchery, and facilities for the breeding of young pheasants and aviaries with outlets where the young pheasant are bred until they are released to the hunting grounds (Pavlović 2004; Đorđević 2012, 2013). To make pheasant production successful and economically justified i.e. to obtain a sufficient number of healthy individuals, in good condition it is necessary to have appropriate facilities with the usage of appropriate production technologies, as well as the continued implementation of biosafety measures defined in biosafety protocol.

Considering that *Salmonella* are the most important cause of bacterial gastroenteritis in humans and are responsible for 24% of food-borne diseases caused by known pathogens in the United States (Tauxe, 2002) its importance is large. Considering the fact that the hunting pheasant is popular in Serbia and that a large number of people consume meat pheasants, we decided to do the experiment which will show of a risk assessment adopted by the presence of brown rat from the point of introduction and spread of *Salmonella* in pheasant farm.

Prevention of entering zoonotic agents in primary production largely depends on the implementation of biosecurity measures on farms. Rodents can largely transmit zoonotic bacteria and bring them to the farm (Gratz, 1994; Leirs 2004).

Salmonella are rod-shaped, motile Gram-negative bacteria of the family *Enterobacteriaceae*. More than 2300 serotypes have been described (Popoff and Le Minor, 1997) most of which are non-host-specific (Jensen et al 2004). *Salmonella* are facultative anaerobes. Bacteria colonies grow on plain agar, at an optimal temperature of 37 ° C.

Control and better elimination of zoonotic pathogens which include also *Salmonella* is a priority of today farm production, which includes the production of pheasants in pheasant farms because it leads to a drop in production and consequently if animal products derived from such animals reach the consumption can transmit the disease to the people.
Besides the risk of serious human *Salmonella* infections in the pheasant can significantly affect the economic viability of farm raising pheasants. The economic losses are reflected in dropping eggs production, death of young pheasants, and the adults are in weak condition with poor exterior.
As the pheasant farms are mostly located in areas outside the village in the rural parts, the risk of rats entering is thus higher, because they are finding ideal conditions for food and habitat within the holding yard. Good zoohygienic and deratization measures can greatly reduce the number of rodents present on the farm, but they can never be completely removed. For this reason, it was necessary to determine the status of *Salmonella* in brown rat in the holding yards in order to see what is the real danger of such entering of *Salmonella* infections in pheasant farms.

Material and Methods

Pheasant farm "RIT on which the experiment is conducted is located near Belgrade in Serbia, outside the settlement. The total area of the farm is 22.4ha. Around the farm there is no fence, in surrounding there are agricultural areas with crops. The space between the facilities and aviaries is relatively arranged with dirt tracks and mown grass. The experiment was conducted from april til june.
Collecting of rodents was carried out using the live catch cage rat traps. Traps were baited with chicken feed and various kinds of grains and seeds. The traps were placed in 3 zones with 4 traps. The first zone was the edges of holding yards, second zone was around the production units and the third zone was in the interior of the production units (Picture 1). Traps were checked every day. In the case that the target rodent caught in the trap, the trap was brought to vivarium where was kept until the beginning of the test (up to 3 days) and the daily food and water were added.

Picture 1



Before dissection, each animal was first stunned with ether, the weight was measured and anesthetized with pentobarbital in a dose of 40-60mg/kg intraperitoneally. Anesthetized animals were measured total length (from tip of the nose to the tip of the tail), the length of

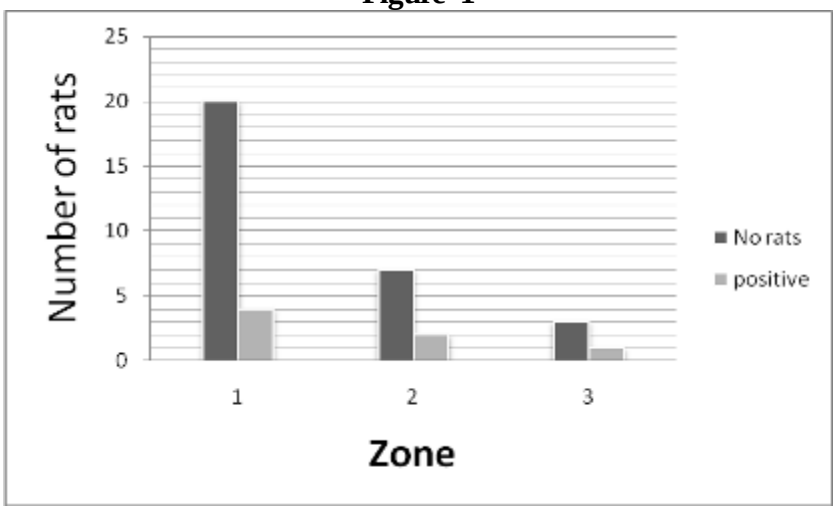
the body with the head and tail length. The animals were tied on the back on dissecting board, limbs were fixed and cut was made along the linea alba side to the sternum. To analyze the presence of *Salmonella* sample of feces was taken from the rear part of colon, and other samples were taken for other analysis which are not the subject of this paper. The corpses of the treated animals were packed in plastic bags and stored in the freezer for that purpose till the transport to the pound where they were destroyed. Individuals were categorized based on the weight in the 3 categories: juvenile with weight up to 100g, subadult with a mass between 100 and 200g, and adults with a mass exceeding 200 g.

For the isolation of *Salmonella spp*. was taken 1 gram of feces from each animal. The sample was primarily enriched on non-selective media Buffered Peptone Water (BPW), and incubated on 37^0C for 18h. After that was done on a selective secondary enrichment media RV (Rappaport-Vassiliadis) is incubated for 24h at $41,5^0$C. The selective isolation and identification was made on xylose lysine deoxycholate (XLD) agar and incubated for 24 hours at 37^0C. Another selective medium was incubated in the accordance with the manufacturer's instructions (BGA, Rambach). Suspicious colonies were transferred on nutrient agar.

Results and discussion

During April and May, a total of 35 individuals of brown rat - *Rattus norvegicus* were caught. Of that number, 19 individuals were male (54.29%) and 16 female individuals (45.71%). The age structure of caught animals was as follows: juvenile individuals were caught 10 (4 females and 6 males), subadult individuals 9 (4 females and 5 males) and 16 adult individuals (8 females and 8 males). Observed by zones from 35 caught individuals 20 were caught in the first zone, 7 in the second and 3 in the third zone, two in the food storage and one in aviary for the parent flock. (Figure 1) Of the total 35 analyzed individuals positive were 7 (20.0%) and these were adult individuals (3 males and 4 female). In all 7 cases was *S. enterica* subsp. *enterica* serovar *Enteritidis*.

Figure 1



We can conclude, considering the total number of caught animals, and the area of the farm that rodents infestation is low. As the largest number of individuals (20) were caught in the first zone, ie. In the edges of holding yards, a fewer in the second zone (7), and in the third zone only (3) shows that the zoohygienic and zootechnic measures in the pheasant farm was well implemented, and all this for reasons of strict adherence to biosecurity protocol since it was introduced three years ago.

Age structure and sex ratio of caught animals corresponding to the status of the wild rodent population for this time of year.
Considering the fact that only 7 of the 35 specimens were positive on *Salmonella enteritidis* and that three individuals caught in the first zone, i.e. the edges of the pheasant farm and one in the third zone, ie. In the aviary for the parent flock we can conclude that the rats in future period, by ensuring continued following of biosafty protocol will not be the way of entering salmonella on farm.

Conclusions

Continuous implementation of biosecurity measures on the basis of defined protocols directly affected the regulation of the number of brown rats in the holding yards and reduce the health risks that potentially infected rats with *Salmonella* by their presence bring in on farms facility. The low level of infestation and the number of positive animals indicates a low level of risk of salmonella infection entering through the brown rat on the farm and the low level of potential disease animals from *Salmonella*.

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STUDY IN VITRO OF HONEY AND ROYAL JELLY AGAINST ENTOMOPATHOGENIC BACTERIA ISOLATED FROM *VARROA DESTRUCTOR*

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Abstract

Many authors have reported studies on antimicrobial effect of honeybee products against clinical microorganisms, but to our knowledge, there is no data about the activity against entomopathogic bacteria isolated from honeybee pest, *Varroa destructor*. Use of chemical acaricides such Fluvalinate and Flumethrine are harmful to bee populations and leaves the residues in honey. Recently, the use of biological agent (*Beauveria, Metharizium*) and bioproducts have shown convincing and satisfactory results in biological control. The aim of this work was to study *in vitro* effect of honeybee products (honey and royal jelly) against bacteria (*Bacillus* sp and *Pseudomonas* sp.*)* isolated from *Varroa destructor*. The products provided from *Apis mellifera intermissa* were collected when the larvae of queen honey bees were 3 days old. The samples were kept frozen at 4° C in dark jars until analysis. For the isolation and identification of the bacteria, the macroscopic and microscopic characters were done according to the Bergey's manual of systematic Bacteriology. Biochemical characteristics were tested by using API 20E galleries (Biomerieux). To test this activity, the agar diffusion method is used. The bacterial isolates were incubated at 37 ° C for 24 h. As a control, we used distiller water. All experiments were made in triplicate and means ± standard deviation were presented. The results obtained show that the honeybee products inhibit the growth of *Bacillus* sp. compared with *Pseudomonas* sp. but royal jelly exhibited the strength activity. The ectoparasite is the vector of many pathogenic agents, which accelerates the death of bees.

Key words: *antibacterial activity, honeybee products, bacteria, Varroa destructor, honeybee.*

Introduction

Varroa destructor is a natural obligate ectoparasitic of Asian bee *Apis cerana* and *Apis mellifera* worlwide. The mite causes damage of bee colonies (Anderson and Trueman, 2000). Several methods have been used to control the pest. Chemical control by the use of Fluvalinate, Flumethrin are harmful to bee populations and can leave the residues in bee products and also *Varroa* can develop resistance to chemical controls (Milani, 1999; Elzen *et al.*,2000). Recently, biological control by *Beauveria bassiana* and *Metharhizium anisopliae* has given satisfactory and promising results as a new means of alternative control (Kanga *et al.*, 2006; Meikle *et al.*, 2007). Few bacteria have been tested for the control of *Varroa destructor* infestation, for example, strains belonging to the Bacillaceae and Micrococcaceae families (Tsagou *et al.*, 2004). Biological control is also ensured by the use of honeybee products against microorganisms. Many researchers have studied the effect of bioproducts against clinical microorganisms (Sahin *et al.*, 2004; Conigli *et al.*, 2013; Elbanna *et al.*, 2014; Isiaka *et al.*, 2015. Belaid *et al.*, 2019) and predator (Lomer *et al.*, 1999; Gende *et al.*, 2008), but a few researches of the antibacterial effect of honeybee products against bee disease are

limited (Antunez *et al.*, 2008; Damiani *et al.*, 2010). This work was the first study which undertaken to find the effect of honeybee products (honey and royal jelly) against bacteria (*Bacillus sp.* and *Pseudomonas sp.)* isolated from *Varroa destructor.*

Material and methods

Samples collection

A total of five samples, three Honey (H1, H2 and H3) and 2 royal jelly (RJ) (J1 and J2) are collected in spring period from the apiary of Tizi Ouzou situated in the northern of Algeria. The samples of RJ provided from *Apis mellifera intermissa* were collected when the larvae of queen honey bees were 3 days old. The samples were kept frozen at 4° C in dark jars until analysis. Samples of adult female mites of *Varroa destructor* were obtained from parasitized brood combs. The experiments were performed in triplicate.

Isolation and identificaion of bacteria from *Varroa destructor*

The bacterial isolates were identified to Bergey's manual of systematic Bacteriology Holt *et al.*, (1994). Biochemical tests were carried by using API 20E galleries (Biomerieux)

Determination of Bacterial activity in vitro

To test the bacterial growth inhibition with the agar diffusion methods was assayed against Gram negative *Pseudomonas sp.* and Gram positive *Bacillus* sp isolated from *V. destructor*. The bacterial isolates were incubated at 37 ° C for 24 h. As a control, we used distiller water.

Results expression

According to Coniglio *et al.*, (2013), the diameter of the inhibition zone (mm) on the plates was measured. The antibacterial activity was determined from the estimation of the diameter of the inhibition zone produced by the samples, following highest activity, diameter > 25 mm; intermediate activity, diameter ≥ 12 mm and ≤ 25 mm; lowest activity, diameter < 12 mm. All experiments were made in triplicate and means ± standard deviation were presented.

Results and discussion

Isolation of entomopathogenic bacteria

According to the Bergey's manual of systematic Bacteriology (Holt *et al.*, 1994), the colonie (S1) Gram positive, motile, coccoid shaped, aero-anaerobis, endospore forming, catalase and oxidase positive identified as *Bacillus* sp was chosen. *Pseudomonas* sp. isolated from *V. destruc*tor (S2) is Gram-negative, oxidase-positive, strict aerobic and non-spore forming.

The biochemical characteristis of the two isolates based on API 20E galleries (Biomerieux) were presented in Table I.

Table I: Biochemical characteristics of isolated bacteria from *V. destructor*.

	S1	S2		S1	S2
Ortho-nitro-phénol-galactosidase (ONPG)	+	-	Mannose (MANE)	-	+
Arginine di-Hydroxylase (ADH)	+	+	Inositol (INO)	-	+
Lysin di-Carboxylase (LDC)	-	+	Sorbitol (SOR)	-	+
Ornithine décarboxylase (ODC)	-	+	Rhamnose (RHA)	+	+
Citrate utilization test (CIT)	+	-	Saccharose (SAC)	+	+
H_2S Production test	-	+	Melibiose (MEL)	+	+
Urease (URE)	-	+	Amydaline (AMY)	-	+
Tryptophane Désaminase (TDA)	-	+	Arabinose (ARA)	+	-
Indol production (IND)	-	-	Oxidase test (OXY)	+	+

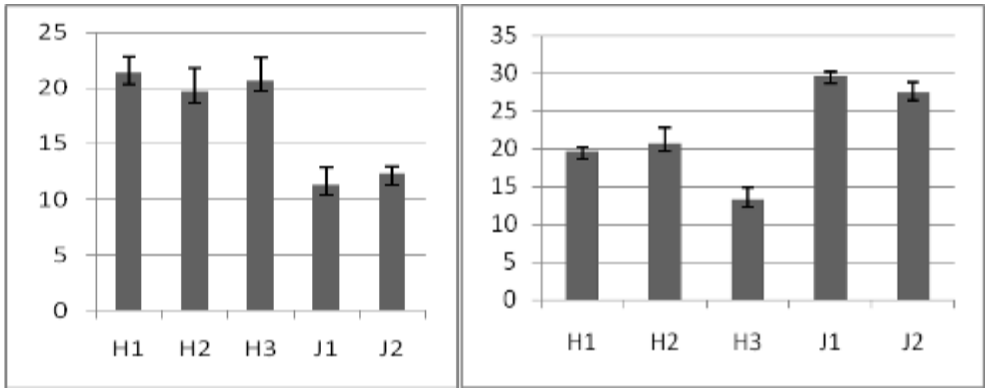
Acetoin production (VP)	+	+	Catalase test (CAT)	+	+
Gelatinase (GEL)	+	-	Motility (MOT)	+	-
Glucose (GL)	+	+	Oxidase	+	+

(+): positive test ;(-): negative test; S1: *Bacillus* sp; S2: *Pseudomonas* sp.

Our preliminary study showed that *Bacillus* sp and *Pseudomonas* sp harbored the parasitic mite *Varroa destructor* collected from Tizi Ouzou. In recent years, microflora of *Varroa destructor* has caught the attention of many researchers. *Bacillus sp*, Micrococcaceae, *Pseudomonas* sp, *Paenibacillus* wynnii, *Burkholderia*, *Pseudomonas syringae* etc… were reported by many authors (Tsagou *et al.*, 2004; Alquisira-Ramírez *et a*l., 2014; Maddaloni and Pascual, 2015; Vanikova *et al.*, 2015). The ectoparasite is the vector of many pathogenic agents, which accelerates the death of bees.

Bacterial activity

Data regarding the antibacterial activity of five indiluted honeys and royal jelly collected in Northern of Algeria against *Pseudomonas sp.*and *Bacillus* sp. isolated from *V. destructor* were given in Figure 1.



a b

Fig. 1. Bacterial activity of honey (H) and royal jelly (J) against *Pseudomonas sp.* (a) and *Bacillus* sp. (b) isolated from *V.destructor*.

The study revealed that the honeybee's product showed the inhibitory zone against the bacteria tested. The highest zone of inhibition was noted with the royal jelly against *Bacillus* sp comparatively to *Pseudomonas* sp. isolated from the honeybee pest. The mean diameter of zones for undiluted RJ samples against *Bacillus sp.* was calculated as 29.66 ± 0.57 mm for Sample J1 and 27.33 ± 1.52 mm for Sample J2. However, our preliminary experiments indicated that all three honey tested showed the best activity against *Pseudomonas* sp. The zone of the inhibition diameter was respectively 21.33 ± 1.52mm; 19.66 ± 2.08 mm and 20.66 ±2.08 mm. The lowest effect against the organism was recorded with the royal jelly (11.33 ± 1.52 mm for J1 and 12.33 ± 0.57 mm for J2).

Adil Ansari and Alexander (2009) found that honey in pure form (with out dilution) was effective against clinical bacteria such *Pseudomonas aeroginosae*, *Escherichia coli* and *Staphylococcus aureus* using the disk diffusion technique. Junie *et al.*, (2016) found that pur honey has a significant antibacterial activity against some bacteria which are resistant to antibiotic (*P.aeroginosae*, *S.aueus*, *S.epidermidis* and *B.subtilis*). A few studies has documented about the efficacity of honey products against bacteria of bees. Antunez *et al.*, (2008) reported that propolis extract present an excellent effect against *Paenibacillus larvae* the causative agent of American Foulbrood, a severe disease that affects larvae of the honeybee. According to Elbanna *et al.*, (2014), the antimicrobial activity of honey is one of the characteristics that make it beneficial to human health. In the study, the bioproduct that

appear more effective to *Bacillus* sp was the royal jelly. Attala *et al*. (2007) found that RJ have the best activity against clinical Gram positif (*Staphylococcus aureus, Bacillus subtilis* and *Listeria monocytogenes)*. According to Bilikova *et al*.,(2001), the RJ had an inhibitory effect against the honeybee pathogen *Paenibacillus larvae*, the primary pathogen of American foulbrood disease, as well as against other Gram-positive bacteria such as *Bacillus subtilis* and *Sarcina lutea*. Barnutiu *et al*., (2011) reported that in the chemical composition of the product, a special exclusive food of the queen honey bee, have been identified a number of antimicrobial peptides including royalisin, apisimin, jelleines I, II, III, IV, 10-Hydroxy-2-decenoic acid (also called 10-HAD or royal jelly acid), apalbumine. Royalisin was found to have potent antibacterial activity against Gram positive bacteria at low concentrations but not against Gram negative bacteria. Royalisin may be involved in a defense system active against bacterial invasion of the honeybee. Biological activities of honey, propolis and royal jelly are mainly attributed to the phenolic compounds such as flavonoids. Flavonoids have been reported to exhibit a wide range of biological activities, including antibacterial, antiviral and anti-inflamatory (Viuda-Martos *et al*., 2008).

Conclusions

The results obtained showed that the honeybee products inhibit the growth of *Bacillus* sp. and *Pseudomonas* sp. but royal jelly exhibited the strength activity against the first organism. Our results showed the importance of the bioproducts in the antibacterial potential and open new perspectives for the study of the biological controls of the pest.

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RURAL DEVELOPMENT AND AGRO-ECONOMY

GOVERNANCE IN AGRICULTURAL COOPERATIVES

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Abstract

The global experience in cooperation and its market indicate that cooperatives hold substantial market shares in agriculture industry–about 80 % in the Netherlands and Finland, about 50 % in Italy and France. At the same time in Latvia, as in other post-communist countries, the development of cooperatives in the agricultural sector is rather low. The aim of the current paper is to determine governance among agricultural cooperatives. In particular, it aims to (1) explain the importance of governance based on scientific research and definitions; (2) identify what governance models are offered by the Latvian Law on Cooperative Societies; and (3) establish what are the governance groups and the key factors of their interrelationships. Legislation analysis indicates that the Latvian Law on Cooperative Societies is based on the German law. Likewise, it is also progressive and promotes cooperation in all respects. However, the legal component is not the determining factor for a successful development of agricultural cooperatives. If market conditions are equal at the macroeconomic level, especially in the Member States of the European Union, then the strength of the cooperatives is to be found at the microeconomic level. According to scientists and business experts, a strong internal environment makes companymore competitive and risk-resistant. As the governance models of Latvian cooperatives do not differ from the governance models of cooperatives in the other countries, the links between these groups (members, board of members, management, etc.), such as trust, interdependencies, legitimacy, transparency, are highly important.

Key words: *governance, agricultural cooperatives.*

Introduction

The OECD and European Commission programming documents identify cooperation as an essential tool for strengthening the competitiveness of small and medium-sized farms along the food chain (OECD, 2019), (European Commission, 2019). Agricultural cooperatives have over hundred years of experience in the neighbouring countries (Finland, Sweden, Denmark) and worldwide while in terms of market the cooperatives held substantial market shares in agriculture industry – 83% in the Netherlands, 79% in Finland, 55% in Italy and 50% in France(European Commission, 2020). From local perspective the Latvian agricultural cooperatives are in stagnation since the number of members has not changed during the past decade although the entire legislative environment is positive in Latvia. About 76% of the total labour input in Latvian agriculture is unpaid family labour. They typically occupy less than 4.9 ha and, altogether, use 2.2% of the utilised agriculture are. The commercial farms structure is dual; livestock farms are typically smaller than the average EU livestock farm, whereas cereal farms are mostly large and export oriented. Cereals are Latvia's top agro-food export commodity group (OECD, 2019). After Soviet Union regime, the first agriculture cooperatives were establish in Years 1992 -1993 in cereals, dairy and vegetable sectors. There were establish 693 agriculture cooperatives during the period 1992- 2019 (Register, 2019). Nowadays there are registered more as 200 agriculture cooperatives. The procedure for the recognition of agricultural cooperatives was introduced since Year 2004. Criteria and

evaluation process is defined in Cabinet of Ministers Regulations(Cabinet of Ministers LR, 2019).

Status of recognition of agricultural cooperative every year get around 50 cooperatives (Figure 1) (Ministry of Agriculture, 2019). If the cooperative has been recognized, it is a kind of guarantee to the farmer that the cooperative can be trusted. In addition, obtaining recognition status gives you the opportunity to receive national and European aid for cooperatives, as well as other statutory benefits.

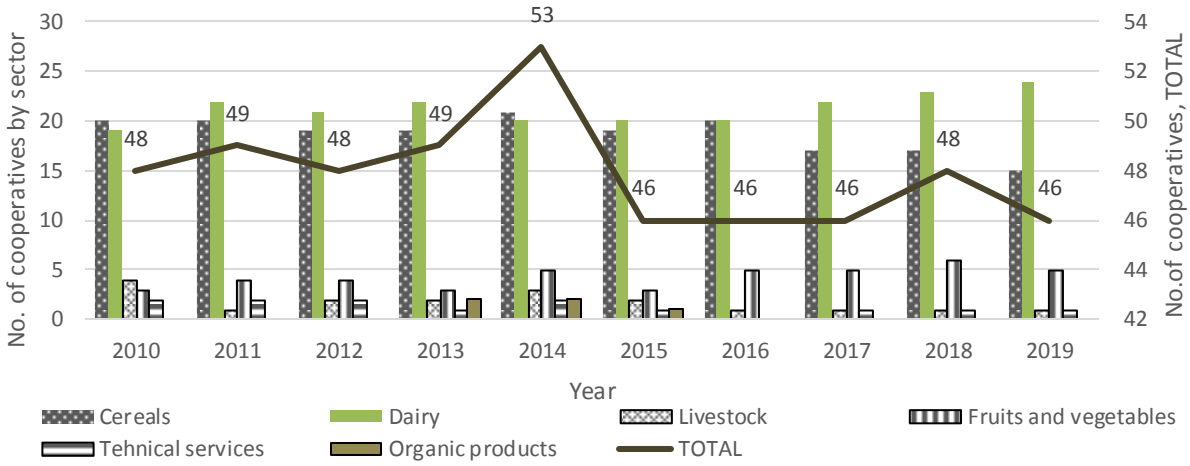


Figure 1. Number of recognized agricultural cooperatives in Latvia, 2010 – 2019

Source: author's calculation based on data of Latvian Association of agriculture cooperatives (LLKA, 2020)

Cooperatives have to adjust and adapt to changing market conditions. Regardless of the country in which they operate, they must be able to compete in the global market. As stronger inclusion of the customer quality requirements is needed for a more robust, cooperative and sound operation for the benefit of the users. It may be necessary to strengthen vertical coordination in the development of customer orientation strategies. As well commitments of members is very important in agricultural cooperatives. How can cooperative leaders use customer orientation strategies smoothly while maintaining the commitment of members (Cechin, Bijman, Pascucci, & Omta, 2013)? To answer this question, one must first disentangle commitment, which requires a framework that links organizational mechanisms to the alignment of interests and actions, that is, to commitment. The authors put forward the legal component is not the determining factor for a successful development of agricultural cooperatives. If market conditions are equal at the macroeconomic level (market prices, demand, etc.), especially in the Member States of the European Union, then the strength of the cooperatives is to be found at the microeconomic level (Iliopoulos, Värnik, Filippi, Võlli, & Laaneväli-Vinokurov, 2019). According to scientists and business experts a strong internal environment makes company more competitive and risk-resistant. The physical sciences indicate that the measurement errors are of the same magnitude as the investigational phenomena, therefore the answer is not to try to squeeze out more information of statistical means; instead, techniques of observing phenomena at a higher resolution need to be found. The appropriate economic strategy is obvious: to provide new types of data at the micro level (Simon, 1984). In other words, the statistics show the overall trend, but to understand better cooperation in agricultural sector there is need to provide new types of data at the micro level. Cooperatives in former Soviet countries have rarely been studied at the micro level and thus organizational design questions seem to have escaped the scrutiny of scholarly research (Hagedorn, 2014). Given the prevailing socio-economic and political conditions in such countries, agricultural cooperatives are expected to play a much wider and important role than in the past (Mõtte, Lillemets, & Värnik, 2019; Iliopoulos, Värnik, Filippi, Võlli, & Laaneväli-

Vinokurov, 2019). The governance of agricultural cooperatives has heretofore received relatively little attention from scholars (Chaddad & Iliopoulos, 2013). So far, there are no known publications on the internal environment of Latvian agricultural cooperatives, on governance structures and their operation. In this article, the reader will get acquainted with international research on governance and the governance structures specified in Latvian legislation.

Materials and methods

This assessment is based on a literature review of journal articles, book chapters and working papers, policy documents and Latvian national legislation. For the implementation of the research purpose monographic data will be used - forming a theoretical discussion; data grouping, analysis and synthesis methods - for information collection, logical arrangement and systematization.

Results and discussion

Governance

From a relational perspective, governance is the "set of relations between the companies' management, its board, members and other interested parties"(OECD, 2004). From a different perspective, it is a structure in which people make decisions on the cooperative performance targets, their implementation and judge whether it has succeeded. The success of a cooperative depends in part on the quality of its governance (Birchall, 2014).

Governance means to improve order, thus reducing conflicts and realizing mutual benefits. It should be seen as a matter of efficiency, combining economics with organizational theory (Williamson, 2014).

Governance identifies the power dependence involved in the relationships between those involved in the collective action. Power dependence means that: (a) the organizations involved in the collective action are dependent on other organizations; (b) in order to achieve their objectives, organizations must exchange resources and agree on common objectives; (c) the outcome of the exchange is determined not only by the resources of the participants but also by the rules of the game and the context of the exchange. The governance perspective deliberately chooses different trends and developments. Its value can be judged by how good or bad the selection has been (Stoker, 2018). Authors agree to G. Stoker highlights that governance is ultimately about creating the conditions for orderly rules and collective action. Therefore, the results of governance do not differ from those of the government, though the processes may be different.

Governance include participatory management, interactive policy making, stakeholder governance, and collaborative management, As a management process, it is rather broad as it likewise encompasses various aspects of the governing process, including planning, policy making, and management (Ansell & Gash, 2008).

Governance models offered by the Latvian Law on Cooperative Societies

Legislation analysis indicates that the Latvian Law on Cooperative Societies is based on the German law, is progressive and promotes cooperation in all respects. A new law on cooperative societies was adopted in 2018. Comparing with the previous version, this is an umbrella law of all kind of cooperative societies. The aim of the respective Law is to create favourable regulatory conditions for cooperatives - voluntary associations of individuals whose purpose is to promote effective implementation of members' common economic interests (LR Saeima, 2018).

The governing bodies of the cooperative are the General Assembly of Members and the Management Board or CEO, as well as the Meeting of Representatives and the Board of Members (if the cooperative has a Board of Members)(LR Saeima, 2018). According to the law, there are four possible types of cooperative governance structures (Figure 2). The General Assembly of Members is the highest governing body of cooperative. Members' General Assembly is replaced by a Meeting of Representatives, if required by the statute and the cooperative has more than 100 members with voting rights. If the cooperative does not form a Board of Members, its functions are performed by the General Assembly of Members. The Management Board or CEO is the cooperative's executive body, which manages and represents the cooperative (LR Saeima, 2018).

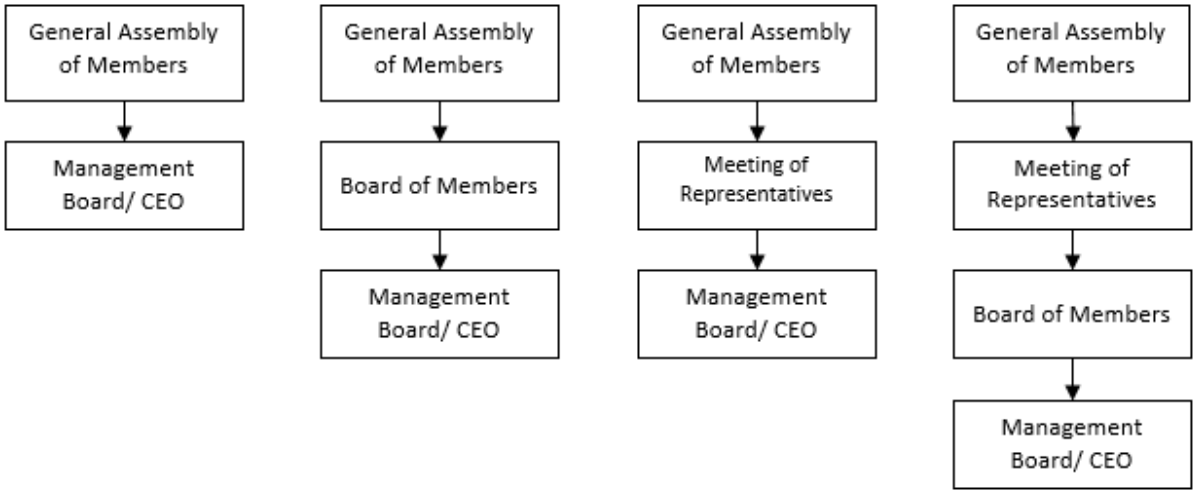


Figure 2. Cooperative governance structures by the Latvian Law on Cooperative Societies
Source: Latvian Law on Cooperative Societies

From governance view the governance structures of Latvian cooperatives do not differ from the governance structures of cooperatives in the other countries around the world. Cooperative itself determined which governance structure to be chosen in accordance with national law of cooperatives and knowledge of cooperative governance (Iliopoulos et al., 2019). But the links between these governance groups (members, board of members, management, etc.) are highly important. (Bijman et al., 2012; Chaddad & Iliopoulos, 2013; Iliopoulos et al., 2019).

Key factors of governance groups interrelationships

The game theory is one component of the neoclassical revival. Game theory addresses the problem that arises whenever an economic participant takes into account possible reactions to other participants' decisions or interdependencies (Simon, 1978). It is a set of analytical tools and solution concepts that provide explanatory and predictable power in interactive decision-making situations where the goals, objectives and choices of the actors involved are potentially conflicting (Szabó & Fáth, 2007).

Governance weaknesses arise when the actors responsible are unable to understand how the various issues and actors are interlinked (Bergsten et al., 2019). How interdependencies of cooperative actors influence on cooperative as a whole is explained by John Nash's invention of the concept of strategic equilibrium for non-cooperative games. Nash equilibrium is a strategy profile in which no player has unilateral incentives to deviate from it if a different strategy is chosen. In other words, Nash equilibrium strategies generates the "best answers" to each other (Szabó & Fáth, 2007).

Game theory promotes an understanding of interdependencies and how the decision of one actor will affect the cooperative as a whole and each of its members. To build strong interdependency links between all actors of a cooperative is highly important.

According to game theory, the values characterizing interdependence are transparency, communication and trust. As mentioned above, governance is a set of internal laws and regulations, procedures and criteria. Therefore, one of the key factors for a strong link between actors is legitimacy. Chester Barnard had underlined that for leadership to be effective, it had to be perceived as legitimate, (Gabor & Mahoney, 2010). In other words, rules and teams only work if the people to whom they are given decide to follow them - they accept the rules and their issuer.

Cooperative growth can create a growing gap between members and their cooperative. In addition, the expanded scope and services create conflicts of interest between stakeholder groups. Therefore cooperatives may face the loss of confidence, and for that reason trust is becoming increasingly important (Jensen-Auvermann, Adams, & Doluschitz, 2018). In general, trust is a key aspect that people use to prevent risk (Massaro, Moro, Aschauer, & Fink, 2019). History is an important factor influencing the institutional environment. Positive experiences of cooperative development usually have resulted in the generation of trust and increase in social capital. Development of cooperatives is influenced by the general situation in the country; a research in the EU Member States shows a clear correlation between the level of general trust as "trust in people" and cooperative performance - all New Member States have low trust and relatively little role of cooperatives (Bijman et al., 2012). In the post-communist countries overcoming the communist legacy, convincing members by building trust, coping with fundamental collective action problems, location in poor or vulnerable regions resemble early stages of cooperative movement (Hagedorn, 2014).

In Latvia's case where the earlier history is highly antagonistic, policy makers or stakeholders should budget time for effective remedial of trust-building. If they cannot justify the necessary time and cost, then they should not embark on a cooperation (Ansell & Gash, 2008). The strength of a cooperative in inducing members' commitment to the organization resides, in principle, in the tendency to involve lower information asymmetries and greater trust in the relationship with farmers than would be the case with investors' owned companies (Beber, Theuvsen, & Otter, 2018).

Transparency is essential not only for the cooperative implementation of the principle of democracy, as democracy of the cooperative is satisfied with a large transparency (Borgström, 2013). It also plays a role in the economic performance of the cooperative, example, an important indicator in ensuring the competitiveness of a cooperative is the quality of the product. The quality assessment of the products produced by honest and reliable members can be achieved under conditions of complete transparency (Fatas, Jimenez-jimenez, & Morales, 2010).

By increasing transparency, clearly defined performance indicators allow members, especially in cooperatives with a large geographically dispersed membership, to monitor governance resourcefully. This is all the more important in cooperatives that adopt management or corporate models with a formal and real delegation of power to professional managers (Chaddad & Iliopoulos, 2013).

Conclusion

Governance is ultimately about creating the conditions for orderly rules and collective action. Governance include participatory management, interactive policy making, stakeholder governance, and collaborative management. It is broader since it also encompasses various aspects of the governing process such as planning, policy making, and management.

Legislation analysis indicates that the Latvian Law on Cooperative Societies is based on the German law. It is likewise progressive and promotes cooperation in all respects.

However, the legal component is not the determining factor for a successful development of agricultural cooperatives. If market conditions are equal at the macroeconomic level, especially in the Member States of the European Union, then the strength of the cooperatives is to be found at the microeconomic level.

As the governance models of Latvian cooperatives do not differ from the other countries, the links between these groups (members, board of members, management, etc.), such as trust, interdependencies, legitimacy, transparency, are highly important.

Cooperatives still are the crucial economic development tool to enhance the community and society better standard of living, this is pilot paper for agricultural cooperatives governance future researches.

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EXPLORING THE POTENTIALS OF CYCLE TOURISM AS A DRIVER FOR RURAL DEVELOPMENT: THE CASE OF WINE ROUTES IN THE MUNICIPALITY OF NEMEA, GREECE

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Abstract

Cycle tourism is now an emerging form of alternative tourism that leads to significant benefits in the areas of sustainable development. Especially, the economic benefits of cycle tourism have greatly attracted the interest of the global tourism industry, policy makers and stakeholders. Hence, there is an ongoing attempt for enhancement of this form, including various policy measures and infrastructure development, giving particular emphasis on rural space. A great contribution will be the integration of cycle tourism into a comprehensive and multidimensional tourism management plan. Greece has not yet shaped the proper conditions for an integrated cycle tourism development, while at the same time the use of bicycles is particularly low. It is promising though that the country's rural network consists of roads with low traffic flow that could be, under specific circumstances, ideal for cycling. In this context and on the basis of building a strong relation between cycling and rural space, this research aims to promote cycle tourism along with the enhancement of the primary sector in the municipality of Nemea. Specifically, the research creates an integrated recreational network, consisting of thematic routes addressed to cycling and walking. These routes connect all the wineries in the municipality as well as archaeological sites, traditional settlements and other points of interest. These routes were determined using Geographic Information Systems (network analysis). Furthermore, this research proposes some policy measures and interventions that can support the development of the network. As a result, Nemea, a rural area pioneering in wine making, is estimated to become an alternative tourism destination, sustaining cycling and traditional products promotion.

Keywords: *Cycle tourism, Rural development, Geographic Information System, Wine routes, Nemea*

Introduction

Tourism is one of the most important sectors of the service economy at a global level (Valeri *et al.,* 2016). The most common form of tourism, until recently, was mass tourism that was mainly developed after 1960s. However, it is known that this form has created serious environmental, social and economic issues questioning its sustainability (Theng *et al.,* 2015). Seasonality is considered one of the most common problems affecting negatively the quality of life in these regions. To overcome this condition, alternative forms of tourism have been promoted and the governments have offered motivations (Andriotis, 2003). In this context, cycle-tourism constitutes a very promising alternative form that can bring prosperity both in urban and rural environment (Bakogiannis *et al.,* 2020). This specific model has been developed in several countries of Europe such as the Netherlands, Denmark, UK, etc. (e.g. Simonsen *et al.,* 1998; Downard and Lumdson, 2001). According to Piket *et al.* (2013) cycle tourism can contribute significantly to rural economies by being an additional tool for regional development. Moreover, they underline that the total economic impact of this form is 44€ billion per year in Europe. The development of a cycle tourism model in a certain area, requires the creation of an efficient and attractive cycling network with routes connecting

significant points in the urban or rural regime. There are many factors that affect the effectiveness of these routes (Lew, 1987). Relevant literature suggests that road characteristics (slope, surface quality, etc.) and features related to natural environment are the main criteria referring to the selection of the routes (Bakogiannis *et al.*, 2020).
Rural areas seem to be a suitable background for the creation of a readable and functional cycling network. Although the population of rural areas has been relatively reduced mainly due to urbanization and migration, the remaining residents give particular emphasis on alternatives activities so as their income will be augmented (MacDonald and Jolliffe, 2003). Chambers (2004) argues that rural tourism is a necessity for these regions. According to Kurdus-Kujawska (2010), the development of rural tourism is dependent on internal and external factors. The former include the provided tourist services such as accommodation, and the latter refer to natural conditions such as attractiveness of the natural environment.
Moreover, there are many outdoor activities that attract the interest of tourists. Among the outdoor activities, wine production has aroused tourist interest as the visitor, in addition to a special experience, comes in contact with the cultural heritage, customs and traditions of the area (Bruwer and Alant, 2009). Indeed, wine tourism is considered an emerging sector due to rising tourist demand (Alonso *et al.*, 2015) being a comparative advantage of the regional area for its development (Conte *et al.*, 2014). The creation of an integrated network of wine routes should take into account features of rural areas such as regional brand image, landscape, attractions, accessibility, climate, infrastructure and proximity, which significantly determine the level of its success and attractiveness (Carlsen, 2004). Except of these factors, the effectiveness of wine routes as a rural development tool requires cooperation among wineries and stakeholders (Gazzola *et al.*, 2018; Hojman & Hunter-Jones, 2012). Furthermore, according to McDonnell and Hall (2008), the number of wineries, the quality of the wine produced and the services provided to visitors play an important role in the development of wine routes. Many researchers have underlined the potential benefits of wine tourism (e.g. Ali-Knight and Charters, 2001). Especially, Getz (2000) mentions the extension of the tourist season and consequently the tackling of seasonality with a positive impact on the economy (income) and employment of the region.
This paper focuses on the development of the Nemea region in Peloponnese, through thematic cycling routes and more specifically the creation of cycling wine routes. The method followed has as an integral component the use of Geographic Information Systems for the determination of the thematic integrated recreation network. This network takes into account various parameters that affect cycling namely; low traffic flows, natural attractiveness, distance and tourist attractions.

Material and methods

The research used both primary and secondary data, thus ensuring a wide view upon the existing situation and towards the proposals. More specifically, the primary data were collected via on-site observations and refer to road network characteristics. In this context, we measured the road width of each road segment within the municipality. Regarding secondary data, we retrieved some of them from the OpenStreetmap platform, which is an open dataset with a great variety of spatial data. Namely, we adopted the geographical location of wineries, points of interest (parks, natural elements) and archaeological sites. Also, we extracted the road network classification. The location of the municipality's settlements was derived from ELSTAT (Hellenic Statistical Authority) and their boundaries from Google maps. We should note that we cross-checked the aforementioned data, via a thorough review of satellite images found in Google earth, in order to be precise and accurate.

The first step of the methodological framework is the investigation of the existing situation, in which we conducted the on-site observation surveys and collected all the secondary data. This step formulates the dataset of the research. Next, the second step is the determination of the thematic routes' network that is addressed to pedestrians and cyclists. It should be noted that it is not necessarily an exclusive infrastructure, but it usually accommodates vehicular traffic as well. These routes are defined via the application of the *shortest path algorithm* in a GIS environment (particularly named "*v. net. allpairs*" in GRASS GIS). This algorithm generates the shortest path between all pairs of nodes (points) in a given network (edges). The main criterion is distance, but it is also possible to introduce a cost function, so that results are closer to reality. In this research, we used as *nodes* the wineries, archaeological sites, settlements and other points of interest and as *edges* the road network of the municipality, excluding the highways. The *cost function* used was the inverse road width value, aiming to encourage the selection of routes with greater values. After the application of the algorithm, we checked the consistency of the routes and made some minor changes manually, in order to ensure an adequate continuity level. This algorithm is a great tool for developing evidence-based networks, thus enhancing objectivity and coping with arbitrary solutions.

Study area: general characteristics

The municipality of Nemea is a rural area, located at Peloponnese region with 6483 residents. It includes several settlements (overall 12) which spread across the borders of the region. The main settlement is Nemea with 3853 inhabitants. Regarding the accessibility of the area, it should be mentioned that Nemea is serviced only by vehicular infrastructure and especially car, as public transport is significantly undermined. As for cycling, we should mention that Nemea does not possess cycling infrastructures either in urban or rural areas. Correspondingly, the activity of cycling in the area is risky as the possibility of road accident is severely high. Hence, this existing situation should be transformed urgently. Concerning land uses, we should state that the municipality is mainly rural, as 72% of the area accommodates agricultural uses and only 28% is urban formations. Furthermore, it should be mentioned that Nemea has a considerable number of wineries and notable historical monuments. Therefore, these sites in combination with sustainable mobility measures could be utilized in order to develop an attractive alternative tourism brand.

Results and discussion

The majority of the proposed thematic routes belong to the provincial road network. However, the absence of great flows' intensity on these roads permits the use of bicycle and walking as well. Another important characteristic of the proposed routes is the diversity of the adjacent land uses, since the attractiveness of the environment (natural or built) has an important role in route choice. Hence, the most usual uses are green spaces, forests, agricultural uses and fields (vineyards) in the rural regime, and residential, commercial and public uses within the settlements. In the area, 26 wineries were found and therefore connected. Indicatively, five routes that constitute the most significant ones, are described below:

Route 1: Sternas – Nemeas Provincial Road in the section from the borders of the Municipality to the settlement of Leontio has a length of 1.6 km, an average slope of 2.5% (uphill) and 2.7% (downhill) and belongs to the tertiary national road network. The land uses are mainly rural areas and specifically vineyards. The road is two-way with one lane in each direction and its width is 8.2 m.

Route 2: Efstathiou Papakonstantinou str. is located in the settlement of Nemea and belongs to the main arterial streets of the Municipality. The section belonging to the thematic route extends from the boundaries of the settlement (beginning of Nemeas-Leontiou Provincial

Road) up to Dimokratias Street. It has a length of 1.83 km and an average slope of 2.8% (uphill) and 2.6% (downhill). It is the most central street of the settlement and possibly of the entire Municipality, since it accommodates various adjacent uses such as commercial activities, leisure shops, petrol stations, services, educational and sports spaces, public spaces and residence. The road is two-way with one lane in each direction, without obvious delineation and an islet. The width of the road varies along the road. Specifically, it ranges from 8.5 to 11.5 m.

Route 3: The National Road of Nemea Railway Station in the section from Nemea to the settlement of Ancient Nemea has a length of 2.77 km, an average slope of 5.6% (uphill) and 6% (downhill) and belongs to the secondary road network. Its adjacent uses are mainly rural areas and green spaces. The road is two-way, with one lane per direction and its total width equals to approx. 11m.

Route 4: Dervenakion str. is located in the settlement of Nemea and belongs to the local road network of the settlement. The section that is part of the thematic wine route, begins from Dimokratias str. and ends at the National Road of Nemea Railway Station, having a length of 1.4 km and an average slope of 4.1% (uphill) and 2.8% (downhill). The land uses vary along the road, thus formulating an interesting canvas. Specifically, the section located within the settlement accommodates basically not only activities such as residence or small shops, but also other notable uses such as public services, workshops, churches, etc., while in the section outside the boundaries of the settlement there are agricultural areas (mainly olive groves).

Route 5: The thematic wine route includes a section of the Nemea-Leontiou provincial road. This section has a length of 0.7 km, an average slope of 2.4% and -4.3% and belongs to the tertiary national road network. The land uses are exclusively agricultural lands (vineyards) and wineries. The road is two-way, with one lane in each direction and its width is about 8-11 m.

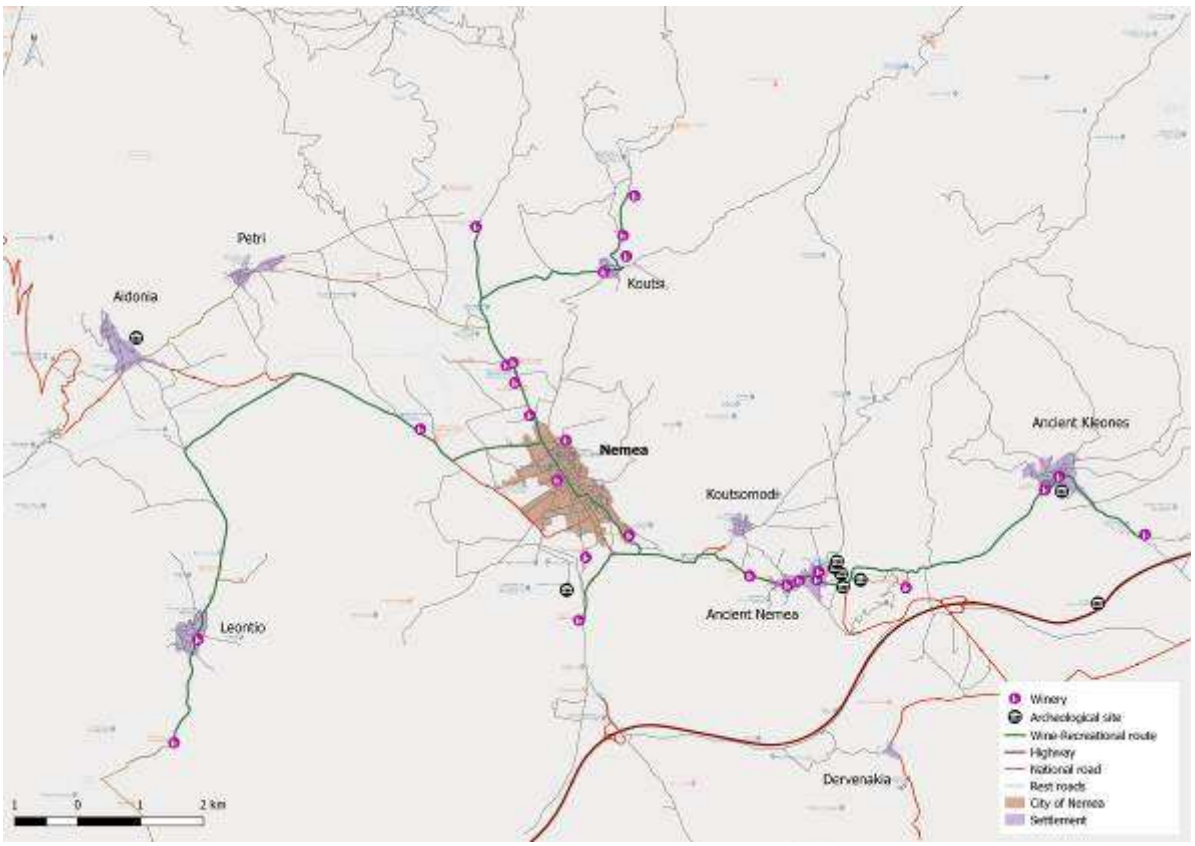


Figure 1. Proposed thematic routes in the Municipality of Nemea

*Source: Authors' elaboration based on observations and secondary data

It is obvious that the routes spread throughout the municipality. The overall length equals to 31,843 km and connects all the wineries of the area. Apart from the wineries, the area has archaeological sites connected via these routes. It is important to mention that these routes link the majority of settlements, offering the opportunity for a competitive and efficient cycle tourist development. This paper attempts to integrate spatial analysis into rural development, and thus promotes and supports cycle tourism for boosting local economy and tourism competitiveness. Specifically, we created an integrated network of recreational routes contributing to the increment of alternative touristic activity in the area. Through this network, the municipality is expected to shape the proper conditions for promoting both cycle and wine tourism. These two forms of alternative tourism could be quite beneficial for the rural development of the municipality. Tellingly, critical development opportunities will arise, thus embarking Nemea into a new era. An era in which the local authorities and stakeholders will invest on both primary and tertiary sector of the economy, and therefore ensure better potentials and an improved quality of life for the residents. In addition, in this new era, Nemea will be transformed into a competitive and attractive alternative tourism destination with national or even European range. One important characteristic of Nemea as a tourism destination is the ability to encourage and promote synergies between different activities and dynamics. For instance, these routes will not only support cycling and walking as recreational activities, but they will also facilitate visits to wineries, archaeological sites and other points of the interest. Furthermore, these routes will eventually enhance the traditional products' market, since tourists will have better chances of visiting and exploring the local stores. Additionally, the routes will also be beneficial for the development of accommodation services, such as hotels, rooms to let, Airbnb, camping sites, etc., in case of vacations that require overnight stays. Hence, it is essential to improve tourism standards, services and infrastructure and cultivate a more supportive environment for investments in rural tourism.
Regarding, the method adopted, we should state that the spatial technique of the shortest path algorithm, improves significantly the accuracy of the results. Focusing on the components of the network, it is worth mentioning that all the routes have the potentials for accommodating cycling and walking. Some of them are already in a good condition, but other present considerable deficiencies. In any case, when the time of implementation comes, the local authorities will decide the proper interventions and policy measures. For instance, they could reduce speed limits, determine cycle tracks and pedestrian lanes, improve traffic junctions, install the appropriate marking and signing, installation of bike sharing stations, etc.

Conclusions

This paper enriches rural development studies by considering spatial dimension, which is often overlooked in relevant research. The utilization of spatial techniques could advance even more the creation of cycling networks, leading to efficient solutions. Notwithstanding, cycle tourism and rural development cannot be fully analysed into a single study. Therefore, we propose the elaboration of new studies that can extend or specify the present findings. In this context, another take would be the creation of a method regarding the design of the routes or a research for tourism preferences concerning combined cycle and wine tourism.

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REALMED PROJECT: SOCIOECONOMIC INSIGHTS AND PROPOSALS FOR MEDITERRANEAN TERRITORIES PROSPERITY

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Abstract

In this paper, we share the results obtained from a socio-economic approach applied in a qualitative case study methodology, about four Mediterranean products: Argan oil from Morocco, *Porco Alentejano* and *Cerdo Ibérico*, from Portugal and Spain respectively, *Djebel* Lamb and Kid from Tunisia, and Truffles from Italy and Slovenia. Those products are connected to vulnerable and specific cultural and environmental hotspots. Due to the high potential market value they can achieve, they may be subject to several frauds and threats. Authenticity and valorisation are then essential engines for the maintenance of local socio-economic activities and of the cultural and natural heritage. We use a variety of data sources, ensuring that we explore the complex issues in a variety of lenses, which may reveal us the associated multiple challenges. We present and integrate all the results we have collected, produced and/or analysed, at the same time explore considerations and insights from other research works, to produce recommendations and best practices, as proposals to apply in future actions. We point out key issues to stimulate rural economies, the exchange and sharing of knowledge and the protection of several stakeholders in the agro-food value chains. Moreover, we propose specific research lines that lead to engage with transdisciplinary methods, highlighting design as a structured working process and a non-technological innovation in the domain of agricultural value chains and applied economy. We consider those prospects vital goals to improve market competitiveness, quality and innovation both in regional, national and global scales.

Keywords: *Innovation, Mediterranean Value Chains, Socio-economic Valorisation, Sustainability.*

Introduction

REALMed project boosts the conservation of Mediterranean (Med) biodiversity and the activation of multi-stakeholder processes for problem solving and sustainable farming practices. Traditional Med products of exceptional quality are potential engines in the maintenance of local socio-economic activities and of the cultural and natural heritage, particularly products protected by geographical indications such as Protected Designation of Origin (PDO) and Protected Geographical Indications (PGI). Those products support economic, social and environmental sustainability, once they generate links between specific territories and specific local resources (Belletti *et al.*, 2015). North and south of the Mediterranean have different environmental and socioeconomic contexts, and so, confrontation between that heterogeneity with several perspectives and approaches, is useful

to understand the global evolution of agro-systems and to promote an integrative and innovative framework that can guarantee both sustainability of those sensitive ecosystems and local livelihoods. Oliveira *et al.* (2019) highlight the need to review the definition of "innovation" applied to agricultural systems to boost rural development. To generate greater innovation, growth for companies and benefits for society, three key actions could be considered to create shared value opportunities: reconceiving products, markets and productivity in the value chains and enabling local cluster development (Porter and Kramer, 2019). These authors affirm that more than a redistribution approach, as in the Fair Trade movement, sharing the value already created, the shared value perspective must focuses in the improvement of farmers' efficiency, yields, product quality and sustainability. Boshkoska *et al.* (2019) stress the need to cross knowledge boundaries in the domain of agriculture value chains. There are still many challenges in network cooperation, online or offline, being the interaction between technology and territories a current priority in order to achieve a collaborative innovation and a creative territorial networking (Covas and Mendonça, 2019). In that sense, design can help to highlight the importance of one strategic thinking applied to develop new ways of intermediation and communication among stakeholders of products and services value chains, reinforcing the trust between producers and consumers (Krucken, 2009). In Andalusia, a study was developed and its results suggest design as an important tool to explore in the agri-food sector, providing both tangible and intangible benefits, being highlighted the design as an important factor of 'dynamisation' (González-Yebra *et al.*, 2019). The potential of design, beyond its identity as a discipline, can be explored and applied as a builder and a facilitator of sharing knowledge in different domains, specifically when we want to interconnect territorial identities as in the Mediterranean scenario (Parente *et al.*, 2018; Vieira, 2018). The objective of this research paper is to obtain insights about the exposed issues in a qualitative understanding, complementing with quantitative data from our exploratory estimations. Our particular focus is to estimate the potential value associated to each cycle of the four product value chains, identifying the main constraints, needs and standpoints.

Materials and methods

Our socio-economic approach started in March 2019, coordinated by *Instituto Nacional de Investigação Agrária e Veterinária* (National Institute for Agricultural and Veterinary Research) in Portugal, being the general purpose a better understanding of the potential contribution of Med traditional products to the rural development. We apply a qualitative case study methodology with an exploratory strategy to analyse how the value is distributed along the respective value chain for each Med traditional product under testing. We selected the specific local of origin for each product, identified local economic key players to interview them, with the support of each country team, to collect the best and possible information. As secondary sources, we used academic theses, official statistics, technical reports from public institutions and other outreach publications, with focus on scientific articles, searching in Web of Science (WoS) and Google Scholar. A stakeholders meeting was held in Portugal, in March 2019, where it was possible to listen and to gather information from different agents, in the *Porco Alentejano* (a native swine Portuguese breed) value chain. Based on official data and considering the total forest areas, a potential area was estimated with *Quercus* trees, relative to the regions where PDO *Porco Alentejano* products are produced and processed: 98% of the national area of holm oak forest and 90% of the national area of cork oak forest. We used primary data from 50 producers and one industry agent. We estimated the potential value creation associated with the *montanheira* (i.e. the value acorn due to the fattening period in free range, where acorn is the main food resource) having as reference the prices

paid to landowners, the rent paid per hectare during the *montanheira* season. Then, using data from the agricultural market information system (available in http://sima.gpp.pt:8080/sima), we calculated the value of the potential animals produced and the respective meat transformation value in hams and shoulders, both PDO products. Concerning the other products, we made interviews scripts, adapted for each context and for the teams from each country. We sent and received them via email, in several loops, between November 2019 and February 2020. In the context of the Argan Oil value chain, according to the data sent by our partner from Morocco, we assume 830,000 ha as the area covered by argan tree and a density between 25 and 150 trees/ha, assuming an average density of 80 trees/ha. In an ideal scenario, we calculated the potential number of trees by the total area, about 66 million trees (66,400,000 trees), and received the respective estimate average amount of fruits, nuts and seeds for that potential number of trees. We also received the average price of argan oil per litre in the level of production /collecting, processing and marketing, respectively of 19€, 50€ and 173€. In the *Djebel* Lamb and Kid value chain analysis, we received data about 91 farmers from the Northwest mountainous regions, Beja, Jendouba and Bizerte. In the context of the truffle value chain analysis, from Slovenia, we didn´t receive primary data and we found scarce secondary information related to truffles market prices. Thus, our results were based on the Italian context, from where we received data from our partners. We estimated the potential national value from official data (table 4), which is unclear concerning the distinction between cultivated and wild truffles. The official data refer the quantity produced in the country by year, the cultivated area and the average prices practiced. We then compare the values obtained with other potential values that we estimate based on the number of officially registered collectors.

Secondary sources were vital for us, as we used and compared our estimates with other works. In this exploratory phase, our focus is on the potential value (in Euros) of the four products in the three main phases of the value chain: production, processing and distribution / marketing. So, we applied our own exploratory approach and we estimated our results in a maximum possible national production scenario / year. Then, we compared our results and considered them coherent in magnitude to those found in the secondary sources.

Results and discussion

For the *Porco Alentejano* value chain, table 1, below, summarizes our results:

Table 1. Estimation of the Portuguese potential related to the *Porco Alentejano* value chain

Average number of Animals	230,123 pigs
Average area of *montado* (hectare, ha) (PDO products in Alentejo region)	845,813 ha
Average value of acorns for *Montanheira* (10^3 €)	13,800 €
Average sales at production level (10^3 €)	141,266 €
Average sales at industry level (10^3 €)	252,376 €

Source: Authors' elaboration based on the questionnaire survey results.

Despite this reality, in the stakeholders meeting in Ourique, Alentejo, it was pointed out that although PDO certifications may allow a differentiation in product quality, they haven´t been able to achieve effectively the initial planned goals. Also, a lack of an effective regulation and a harmonization of legislation between Portugal and Spain were highlighted, as well the need to improve the communication to the consumers. In addition, stakeholders pointed out the path of innovation, mainly due to the need of a greater organization at different levels, which can be better supported by public policies. So, it would be important to improve communication, the engagement with citizens and stakeholders, building trust and legitimacy

of evidence to apply in policymaking. The sustainability of the acorn production is vital for the entire value chain dynamics. Taking into account the decline of *Quercus* trees, due to phytosanitary and edaphoclimatic problems, among other complex issues, more investments focused on the management of this unique worldwide ecosystem should be a top priority.
In the context of the Argan Oil value chain, we found great variability in several studies, regarding the edaphoclimatic conditions of each specific locality, total area of *Argania* trees in the southwest of Morocco, densities and the productivity of each tree, and other pressures, thus affecting the respectively profitability expressed in litres of argan oil per hectare. The table 2 presents our main exploratory results.

Table 2. Estimation of the Moroccan potential related to the Argan Oil value chain

Average amount of fruits (10^3 kg and kg/ha))	360,465 kg; 434 kg/ha
Average amount of nuts (10^3 kg and kg/ha)	212,674 kg ; 256 kg/ha
Average amount of seeds (10^3 kg and kg/ha))	21,628 kg ; 26 kg/ha
Argan oil yield (10^3 litres and litres/ha)	10,300 litres ; 12.40 litres / ha
Average sales at production level (10^3 €)	195,700 €
Average sales at procession level (10^3 €)	515,000 €
Average sales at marketing level (10^3 €)	1,781,900 €

Source: Authors' elaboration based on the questionnaire survey results.

Within this scenario, we point out the main challenges considering the works from Aubert *et al.* (2015) and Arrahmouni *et al.* (2018). Although the argan oil value chain offers promising prospects in terms of job creation, women's empowerment and promotion of sustainable local development, significant challenges remain to be addressed, both in terms of its various activities as the collection of raw materials, production and marketing, and also at the level of its governance. The area occupied by the argan grove has decreased, a result of the development of intensive agricultural policies in the plain of Souss-Massa-Draa. Faced with these changes in land use, the argan sector has not been a suitable economic lever to counter these dynamics. The ecosystem remains threatened today and it is essential to put the issue of environmental protection at the heart of the development of the sector.
Below, table 3 shows the results related to the *Djebel* Lamb and Kid value chain analysis:

Table 3. Estimation of the Tunisian sample potential related to the *Djebel* Lamb value chain

Average number of lambs / farm	25
Total number of lambs / 91 farms	2,275
Average price at production / farm (€)	1,250 € (50 € / lamb)
Average price at commercialization / sheep in high season of religious festival (€)	175 € / lamb
Average Revenue from Lambs Sales / Production Cycle (€) / 91 farms	113,750 €
Average Revenue from Lambs Sales / Distribution Cycle (€) / 91 farms	398,125 €

Source: Authors' elaboration based on the questionnaire survey results

Although the sample is not representative, since around 5,000 estimated farmers are involved in *Djebel* lamb production in northwest Tunisia, we can estimate the associated potential. Sheep breeding is a major source of livelihood and contributes to the subsistance of landless, smallholder and marginal farmers. However, Tunisian breeders are not yet organized in one association or a group of breeders, being one strategy to apply, officially, the inherent organization. Although there is an ample knowledge in several agricultural areas developed in Tunisia, Blom-Zandstra *et al.* (2017) stress the need for a market driven approach, a farmer-inclusive agri-business development, organizing farmers towards an efficient market orientation.
Finally, table 4 presents the results related to the Truffles value chain analysis in Italy:

Table 4. Estimation of the Italian potential related to the Truffles value chain

Average annual yield (kg / ha)	90,000 kg
Average cultivated area (ha)	1,200 ha
Average price at production level (€ / kg)	1,000 € / kg
Average price at processing level (€/kg))	1,300 € / kg
Average price at marketing level (€/kg)	2,100 € / kg
Average sales at production level (10^3 €)	90,000 €
Average sales at processing level (10^3 €)	117,000 €
Average sales at marketing level (10^3 €)	189,000 €

Source: Author's elaboration based on the questionnaire survey results.

There is an unclear reality of the wild truffle harvest in Italy. To exemplify it, we made an exercise from the official data of licensed truffle pickers According to Furlani (2015), we can consider 63,906 registered truffle pickers all through Italy. This national census of truffle pickers was a first step to better define the truffle value chain, an important basis to develop larger and more complex studies in the future. Just hypothesizing that each truffle picker harvests 10 kg/year of *Tuber spp.*, we yield a yearly amount of free harvest equal to 639 tonnes by year, which is almost seven times the official production data of 90 tonnes by year.

Conclusions

Our results may be useful to scholars, regulators and several actors, not only to understand the national potential of each product by country and the value distribution along each value chain, but also the nature of challenges and prospects of the studied four value chains. However, they are subject to several limitations, as the inherent heterogeneity and complexity of relationships among stakeholders, the different formal and informal market conditions, among other complex factors, being not possible to fully control them. We consider our study a work in progress and a general diagnostic to improve deeper and collaborative studies. Due to the difficulties of reconciling availability for face-to-face meetings with experts from various areas, economic agents and others' stakeholders, we point out the need to extend our effort to further works. There are many environmental and socio-economic challenges to face and it is vital being able to achieve, the most possible, an effective and efficient balance between the global pressures and local responses, particularly in disadvantaged areas and try to better interconnect the forest development and agricultural practices. Within the value chains analysis and applied agriculture economics, we do believe that design, as a structured and personalized working process, can be a strong improver to knowledge transfer, stimulating linkages and harmonization of different knowledge domains. Since data availability has strongly increased accompanying the vertiginous complexity associated to the stakeholders' relationships in value chains, we point out the need to engage with transdisciplinary methods, refining existing ontology models and developing decision support systems. An effective knowledge management will lead to a more robust and fit-for-purpose evidence for all stakeholders, especially when we want to stimulate win-win conditions between them. It will be vital to find key drivers to face globalization, to look for an effective international cooperation and the sharing of sustainable solutions, for each context. The innovation of agriculture systems management is crucial for the adaptation to economic, social and environmental requirements, as the capacity to implement new practices according to the Agricultural Knowledge and Innovation System (AKIS) framework. Considering the great influence of agricultural and food policies on the governance patterns of value chains, we highlight the need to improve two key skills: synthesizing research and managing expert and non-expert communities. Those skills may consolidate interfaces between science and policy, improving the influence of evidence on policymaking.

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EVALUATION OF THE ECONOMIC GAINS OF THE BEE COLONY REMOTE MONITORING

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Abstract

Precision Beekeeping is focusing on individual bee colony remote monitoring using different measurement systems and sensors. One task of the Precision Beekeeping is to continuously collect real-time data about bee colonies for its further analysis with aim to identify different states and abnormal behaviour. In many cases bee colonies have many different parameters available for constant monitoring and the beekeeper should decide what kind of IT system is needed to accomplish this task. Automatic monitoring systems offer many advantages for the beekeepers, but also have their installation price and maintenance costs, so the economic aspect of the IT system implementation should be considered when implementing monitoring system within the Precision Beekeeping. This paper describes possible economic gains while such systems are implemented. Different scenarios are observed and discussed. Return of investment coefficient is calculated as well to find out how fast investments for system implementation of the specific monitoring system will be returned. In addition, to ease the process of calculation of all formulas and evaluate the economic gains of the implementation of bee colony remote monitoring system, online web tool (application) is developed and published for public use. Based on made calculations and assumptions it can be concluded that beekeepers need adjustable bee colony monitoring system for cheap basic measurements of all colonies and one main module for in-deep monitoring of referenced colony within one remote apiary.

Keywords: *Precision Beekeeping, Precision Apiculture, economics of beekeeping, return of investments, SAMS project.*

Introduction

Beekeeping is traditional and very old branch of agriculture with significant impact on other agricultural sectors, as honey bees are the main insect pollinators. Up to 75% of the crops, used for human feeding depends on pollination (Ollerton et al., 2011; Potts et al., 2016). Bees plays the important role in whole crop production chain and survival of wild plant species (Gallai et al., 2008; Klein et al., 2007). Over the last 60 years dependence on bees for pollination increased to about 300% (Aizen & Harder, 2009). Health status and decrease of the honey bee population worldwide is a growing concern amongst scientists, ecologists, environment specialists, farmers and also a policy makers. It is concluded, that bees are the most impacted species by the anthropogenic factors (application of agri-chemicals, environmental pollution, changes in land use patterns, Colony Collapse Disorder, etc.) around the planet with serious long-term consequences for agriculture and forestry (Kaplan, 2008). A significant decrease of honey bee colonies can affect the whole agricultural production chain.
In a traditional approach, to evaluate the status of the bee colony, beekeepers have to make frequent visual observation of the bee colony, by opening the hive. Often intrusive bee colony inspections are time consuming, can lead to a stress of the colony (Zacepins et al., 2016) also

remote monitoring reduces beekeeper's spending on unneeded travelling to remote apiary (Komasilovs et al., 2019). Rapid development of the sensing, data transmission, microprocessor technologies allowed the development of the automatic remote bee colony monitoring systems which can assist beekeepers with adding important information on the bee behaviour and possible state without an invasive inspection (Bromenshenk et al., 2015; Human & Brodschneider, 2013; Kridi et al., 2014; Kridi, et al., 2016; Meikle & Holst, 2015; Sánchez et al., 2015; Zogovic et al., 2017).

Future of the traditional beekeeping is to implement smart apiary management approach and start to use automatic and remote tools for bee colony monitoring together with beehive control mechanisms to improve the bee colony productivity. Implementation of technological inventions for the bee colony monitoring lead to the Precision Beekeeping or Precision Apiculture foundation. Precision beekeeping is defined as an apiary management strategy based on the monitoring of individual bee colonies to minimise resource consumption and maximise the productivity of bees (Zacepins et al., 2015). The main idea of the precision beekeeping is to observe each hive individually and make inspections, treatment, management actions only to individual hives, thus minimising the time needed for manual inspections of all colonies and minimising the disturbance of bee colonies. There are many positive aspects of the bee colony remote monitoring with regards of non-disturbance of bee hives, decreasing the production costs, decrease the burden of death rate, detection of the bee colony swarming and increase of the whole bee hive production (Zacepins et al., 2020).

Authors of this paper would like to discuss and evaluate the economic aspect of the remote bee colony monitoring system implementation to the beekeeping practice. Specific aim of this paper is to present an approach for evaluation of the economic gains of the bee colony remote monitoring system implementation for real-time and remote bee colony monitoring procedures.

Material and methods

This section describes approach and methods used for the evaluation of the economic gains of the bee colony remote monitoring. This section describes the basic beekeeping expenses by taking the Latvian beekeeper as an example. Different used variables are defined and assumed either on literature study or available resources, either on individual beekeeper knowledge.

For the evaluation of possible benefits by implementing the remote bee colony monitoring, possible amount of EUR values are compared. As well return of investment (ROI) coefficient is calculated to show the possible outcome of the investments of the monitoring system. Different monitoring system configuration scenarios are compared to demonstrate how the profit and installation costs changes.

Description of basic beekeeping expenses

To evaluate the economic gains of the bee colony remote monitoring it is necessary to understand which expenses positions/situations can be affected by the IT system implementation. At the beginning authors would like to describe the overall situation of the hobbyist beekeeper in Latvia (should be similar also to other beekeepers in other countries) and identify its essential expenses. These costs will be used for further comparison and evaluation. Cost are valid for observed cases but may differ between beekeepers. Some numbers are taken into consideration based on beekeeper's advice and/or based on literature studies. Usually beekeepers are not placing more than 20 bee hives in one location, so authors consider that small hobbyist beekeeper have 20 bee colonies (variable: *NumOfCol*) located in one remote location. Distance to the apiary mostly are not more than 50km. Average honey production per bee colony is considered to be 25kg (variable: *Prodcol*), based on beekeeper's

survey, made by Latvian beekeeper's association (http://www.strops.lv/index.php/raksti/dravosanas-panemieni/467-lbb-biskopju-aptaujas-rezultatu-apkopojums). Price per 1kg of honey is considered to be 4.50 EUR (variable: *Phoney*), (based on http://laukos.la.lv/biskopji-spej-tik-medu-sviest). For basic income (variable: INC_{bas}) calculation following formula is used:

INC_{bas} =NumOfCol*Prodcol*Phoney = 2250 EUR.

Authors would like to define basic beekeeper expenses positions. Authors in this paper only assume positions which potentially can be affected by the implementation of the remote monitoring systems and expenses for equipment, medicine for the bee colonies, etc. are not considered as IT systems will definitely not affect them.

First considered expenses are costs for the remote apiary inspections. These costs can be calculated by taking into account distance to the apiary and wage costs for workers, so expenses consist of two parts: one related to travel costs including car fuel consumption and second related to labour costs. To calculate expenses related to the fuel consumption the distance to the apiary is needed, car fuel consumption per 100km and fuel price. As an alternative, constant costs for kilometre allowance can be used for calculations. For labour related expenses, time for inspection, time for travelling to apiary and person (or persons) wage should be considered. If beekeeper do not want to consider the time spent for work, labour related costs are zero. We propose the following formula for calculation of the expenses to reach the apiary and make the inspection. Default values for variables are also defined:

$Exp_{inspection} = Exp_{fuel} + Exp_{pers}$, where

$Exp_{fuel} = Dist*Fuel_{cons}/100*Fuel_{price}$

Dist = Distance to the apiary (km) = 50 km

$Fuel_{cons}$ = Car fuel consumption to 100km (L) = 8 L

$Fuel_{price}$ = Price of one Liter fuel = 1.20 EUR

$Exp_{pers} = \sum_i^n (Perstime * Pershour)$, where

n = Number of persons going to the apiary = 1 pers

$Pers_{time}$ = Time needed to make a whole inspection (including travelling time) = 120 min for travelling + 200 min for inspection (10min*20).

$Pers_{hour}$ = Salary for one person hour = 5.36 EUR

Costs for one inspection based on provided variables can be calculated as follows:

$Exp_{inspection}$ = (100*8/100*1.2 + 1*320/60*5.36) = 9.6 + 28.59 = 38.19 EUR

It is assumed that beekeepers in average are making 12 inspections (variable: *NumOfInsp*) during the year and thus overall costs for inspection during the year can be calculated as follows:

$Exp_{inspections} = Exp_{inspection} * NumOfInsp$

Yearly expenses for observed case would be: $Exp_{inspections}$ = 38.19 * 12 = 458.24 EUR

Next aspect that generate loses for the beekeeper is death/disappearing/Colony collapse disorder of the colony (variable: Expdeath): Expdeath = NumOfCol*Ratedeath*Pcolony

Authors assume value of one colony death equal to 140 EUR (variable: Pcolony). Average bee colony death rate is 20% (variable: Ratedeath), which results in death of 4 colonies and lead to losses of 560 EUR.

The last aspect which is considered for the calculations are expenses, generated by the swarming of the colonies. Average swarming rate is 10% (variable: Rateswarm), which results in swarming of 2 colonies and lead to losses of 212.50 EUR, assuming, that value of one swarmed colony is 106.25 EUR (variable: Pswarm) (A. Zacepins et al., 2020).

$Exp_{swarm} = NumOfCol*Rate_{swarm}*P_{swarm}$

Summarising all mentioned expenses positions the total expenses can be calculated:

Exp_{total} = 458.24 + 560 + 212.50 = 1230.74 EUR

So basic profit (variable: Profit) for the beekeeper is:
$Profit = INC_{bas} - Exp_{total} = 2250 - 1230.74 = 1019.26$ EUR

Results and discussion

As a result of this research and scientific paper are:

- proposed model (algorithm) for evaluation of benefits by implementing the remote bee colony monitoring system;
- assessment of the ROI coefficient which includes costs of the monitoring system itself, installation and maintenance costs;
- comparison of expenses and benefits of different configuration of bee colony monitoring systems;
- developed Web application to ease the evaluation of the economic gains of the bee colony remote monitoring.

Evaluation of possible benefits by implementing the remote bee colony monitoring

By implementing IT system for the bee colony remote monitoring beekeeper can significantly decrease the number of on-site inspections, thus minimising the costs for going to the remote apiary. It can be achieved by the real-time colony status monitoring and notifications if on-site actions are required.

Apiary inspections could be divided in two groups: inspections that are mandatory on-site (cannot be substituted by any remote methods) and those can be replaced with remote monitoring system. Mandatory on-site inspections are: after winter inspection, adding supers, honey harvest, varroa treatment during summer and varroa treatment during winter. And the second group are inspections to monitor colony productivity and status, like checking if there is enough winter feed, is there a nectar flow and bee health status. Those inspections can be substituted by the remote system, which is able to monitor those situations. For example, nectar flow can be easily monitored by the scales system, activity can be checked by temperature/sound, winter food consumption by scales/temperature. Number of inspections in second group depends on the individual beekeeper and on beekeeping approach, but authors assume that this number on average is 5 inspections per year (based on individual interviews with the beekeepers). As a result, beekeeper can save up to 5 (variable: *NumOfInsp*) visits to the apiary during a year, thus having savings: 5 * 38.19 = 190.95 EUR.

Additional benefit of the real-time bee colony monitoring can be minimisation of the bee colony death rate by instant alarms and continuous bee colony monitoring. Potential decrease of death rate depends from actions taken after receiving the alarm notice. Based on https://apistech.eu/en/hive-monitor-2/ decrease of 20% can be achieved. So, the number of colonies dying each year can be decreased by 20%, thus saving can be calculated as: Round (4 * 0.2) * 140 - 38.19= 101.81 EUR.

Notifications from monitoring system can warn about upcoming swarming event. Economic benefit of one colony swarming (variable: *BnfOfSwarming*) detection is evaluated in another authors paper (A. Zacepins et al., 2020) and that approach can be used to calculate benefits of one bee colony swarming detection. Thus, saving of additional 150.42 EUR will happen.

Location, weather and other factors determine volumes of produced honey. Monitoring system provide data for decisions and actions that potentially can lead to increased production volumes. According to literature and commercial bee colony monitoring system web site (https://apistech.eu/en/hive-monitor-2/) it is evaluated, that increase up to 25% can occur. Total benefits can be calculated from average yearly honey production volume and price per litre.

$Bnf_{prod} = NumOfCol*Prod*0.25*P_{honey}$, where

NumOfCol = number of colonies
Prod = Amount of production in kg (25kg average)
P_{honey} = Honey price per kg (4.50 EUR)
Bnf_{prod} = 20*25*0.25*4.50 = 562.50 EUR
Summarising all calculations, overall benefits expressed in EUR per year of remote monitoring system can be as follows: $Bnf_{sys} = Bnf_{inspection} + Bnf_{death} + Bnf_{swarming} + Bnf_{prod}$
Bnf_{sys} = 190.95 + 101.85 + 150.42 + 562.50 = 1005.72 EUR
According to these calculations, implementation of IT system gives additional economic benefit by reducing potential loses. To calculate ROI of monitoring system, the total costs of ownership (TCO) must be calculated first. TCO expenses include IT system purchase, installation and maintenance costs.

Overview of the commercial bee colony monitoring systems

To evaluate the economic gains of bee colony monitoring system the price of such system, its operation and maintenance costs must be known. Today on the market there are various different bee colony monitoring and measurement systems available. Authors aim is not to name and review all of them, but choice is based on random search results. As a requirement for the system is ability to measure at least temperature and weight of the bee colony with an option to remotely access the data. Some examples of available systems are:

- https://www.wolf-waagen.de / price: 899 EUR per device, 24 EUR/year for software, 15 EUR/year for data transmission
- www.bienenwaage.de Capaz GSM 200 / price: 1310 EUR per device, additional costs for data transmission
- http://www.arnia.co.uk/hive-scales/ / price: 380 EUR per device, 5 EUR/month for data subscription
- https://pollenity.com/product/beebot/ / price: 315 EUR per device, 189 EUR for additional device without scales
- https://solutionbee.com/ /SolutionBee HM-5 Hive Monitor / price: 290 EUR
- https://broodminder.com/ BroodMinder-Citizen Science Kit/ price: 289 EUR

It can be concluded that minimal price for the bee colony system for weight and temperature monitoring is around 289 EUR per unit/hive. Based on previous calculations, benefit of such system implementation is 1005.72 EUR per year. But now considering system costs, the ROI can be calculated as follows:

Costs = 20 * 289 = 5780 EUR (to deploy system for all hives) and
ROI = 5780 / 1005.72 = 5.75 years

Based on provided calculations it can be concluded, that it is not economically feasible to install such system on all hives, as ROI equal to such huge number is not feasible for the beekeepers. Based on consultations with the beekeepers it is concluded, that usually advanced beekeepers install only one system per apiary (per remote location) and monitors weight of one target colony, assuming that other colonies would behave almost similar. Such approach can provide benefit of production increase of approximately 15% and evaluating it in EUR:
$Bnf_{prod} = NumOfCol*Prod*0.15*P_{honey}$ = 20 * 25 * 0.15 * 4.50 = 337.5 EUR
ROI in this case would be 0.86 years, that is a good achievement, but all benefits related to decrease of the death rate, decrease of inspections and minimisation of swarming also disappear in this case.
Authors thoughts are that the best option for the beekeepers to fully utilize the potential of monitoring system and get all the benefits is to use custom configuration of the devices for bee colony real-time remote monitoring. In this configuration there would be one main automatic device to monitor weight changes installed on one control hive, but other hives would be equipped with temperature and/or sound/vibration sensors for swarming detection

and constant monitoring (Aleksejs Zacepins et al., 2016). Connection of sensors can be wired or wireless. Then beekeeper will gain all benefits of swarming detection, minimisation of death and increase in production. In that case system installation costs would be up to 500 EUR (300 EUR main module, 10 EUR per hive additional costs), but benefits would be 1005.72 EUR (as calculated previously). In that way, the period of return of investment would be within one year.

Table 1 below summarises all calculations and gives overview of benefits using different system configurations:

Used variables are:

- NumOfHives - 20
- PriceHoney - 4.5 EUR
- InspectionCost - 38.19 EUR
- Value of the dead colony - 140 EUR
- Swarming value - 106.25

Table 1. Comparison of expenses with different system configurations

	Without IT system	All hives with measuring system	One hive with system	Apiary with custom config
Production per hive	25	31.25	28.75	30
Basic income	2250	2812.50	2587.50	2700
Expenses				
Number of inspections	12	7	12	7
$EXP_{inspections}$	458.24	267.31	458.24	267.31
Number of dead colonies	4	3	4	3
EXP_{dead}	560	458.19	560	458.19
Number of swarmings	2	0	2	0
$EXP_{swarming}$	212.50	0	212.50	0
EXP_{total}	1230.74	725.50	1230.74	725.50
Profit and system installation costs				
Profit	1019.26	2087.00	1356.76	1974.50
Installation costs	-	5780	289	500

Web application to ease evaluation of the economic gains of the bee colony remote monitoring

To ease the process of calculation of all formulas and evaluate the economic gains of the implementation of bee colony remote monitoring system, online web tool (application) is developed and published for public use (https://sams.science.itf.llu.lv/system-eval/). Calculator is pre-filled with default values, and user can change them to adapt for local requirements (needs, peculiarities) and prices (costs). The web tool was built as a single page application using Spring Boot 2 framework (forming back-end) and Angular 6 framework with Bootstrap 4 library to create the client side (front-end) of the application. Result is calculated simultaneously as soon as the required fields are filled. A screenshot of the developed web tool is shown in Figure 1 below:

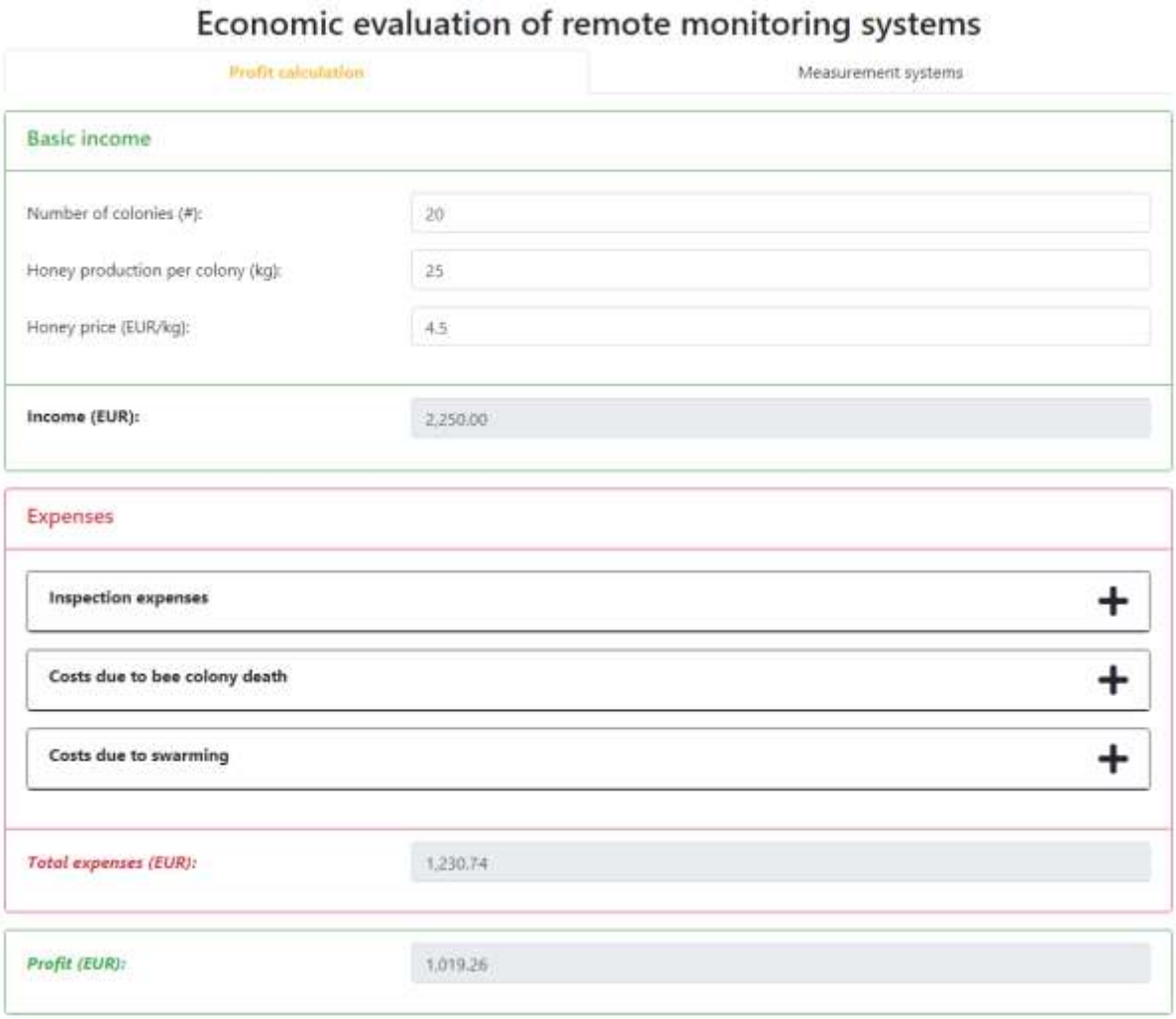


Figure 1. Demonstration of the developed web tool for calculations

Conclusions

The advantage of Precision Beekeeping is the possibility to detect changes or problems in the bee colonies at an early stage giving the beekeeper the possibility to take countermeasures to save bee colonies.

Modern beekeeping and smart apiary management cannot be done without application of information and communication technologies, but each individual beekeeper should specify

what systems he needs most. Implemented systems should minimize the number of on-site inspections of colonies, and maximize colony health and productivity.
Based on made calculations and assumptions it can be concluded that beekeepers need configurable bee colony monitoring system with cheap measurements of all colonies and one basic module for in-deep monitoring of referenced colony within one remote apiary.
To improve the precision of proposed economic evaluation in long term, system repair and additional maintenance costs should be considered.

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COMMUNICATION, DISSEMINATION, AND THE USE OF SOCIAL MEDIA IN H2020 THEMATIC NETWORKS

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Abstract

In order to face new agricultural and forestry challenges, the European Agricultural Knowledge and Innovation System (AKIS) is gaining considerable attention in the European Innovation Partnership for Agricultural Productivity and Sustainability (EIP-AGRI) and hence the European Common Agricultural Policy framework. AKIS aims to bring together various actors, bridging the gap between research and practice to accelerate innovation. Fostering innovation was promoted by the European Union through the development of Thematic Networks (TNs) that aim to collect and share best practices and research results with farmers and foresters in order to exchange new knowledge and innovation for practical uptake. Two important components of fostering innovation in TNs are communication, which aims to promote the project content, and dissemination, which discloses the results. This paper presents commonly used modes of communication and dissemination (C&D) based on an analysis of 28 state-of-the-art TNs within the EURAKNOS H2020 project. The analysis was performed after a desk-top study (investigation of TNs websites), as well as online and face-to-face interviews. Although all TNs applied C&D activities, some TNs do not clearly distinguish communication from dissemination. The results also suggest that, in general, social media enables a good engagement with the end-users, although not all end-user groups are effectively targeted. The observations made in this paper can be used by future TNs to perform efficient and impactful C&D activities. In turn, this may lead to increased exploitation of project outcomes, and thereby accelerate agricultural and forestry innovation and contribute to rural development.

Keywords: *Agriculture, Forestry,Innovation, Result uptake, AKIS, EURAKNOS.*

Introduction

Securing food production in the face of a growing world food demand and ensuring sustainable management of natural resources are some of the main challenges that agriculture and forestry are facing nowadays (Poppe et al. 2009) and should be developed through innovations based on the latest research findings and practice knowledge. To tackle these challenges and foster transition through the agricultural innovation development, the Agricultural Knowledge and Innovation System (AKIS) was implemented and reactivated by the European Commission who also developed the European Innovation Partnership (EIP-AGRI) (EU SCAR 2013; European Commission 2014). EIP-AGRI aims to implement new ways of collaboration among actors, fostering the innovation system approach (Dockès, et al 2011). Moreover, farmers and foresters together with researchers are key actors in the enhancement innovation in the environmental sector. Furthermore, AKIS stimulates the peer-to-peer exchange of knowledge, co-creating innovative solutions to meet the needs of farmers and foresters at field level (European Commission 2019). The core idea of the EIP-AGRI is to

encourage the creation and facilitation of knowledge between farmers, researchers, advisors, NGOs, etc., through the development of the following instruments: Operational Groups (OGs) linked to the Pillar II of the CAP, Focus Groups (FGs) developed by the EIP-AGRI as well as Multi-Actor projects (MAPs), and Thematic Networks (TNs) associated to the EU H2020 research programme (EIP-AGRI 2016). The main characteristic of these EIP-AGRI instruments is that end-users are directly involved in the project's activities, and final results are all oriented to produce ready-to-practice solutions, creating a stronger link between all the AKIS actors and fostering the implementation of latest research findings. To intensify and strengthen project objectives and results, communication and dissemination (C&D) activities are fundamental elements.

This paper focuses on the evolution of the C&D strategies and tools of approved TNs. TNs aim to collect existing knowledge and best practices on a given theme, to make information available in an easily-understandable format for end-users. This creates an opportunity for creative thinking and action, but also requires a clear understanding of the importance of good communication and effective dissemination strategies, also implementing the use of social media (Jaakonmäki, Müller, and vom Brocke 2017).

According to the European Commission (2015), the definition of communication and dissemination is presented as follows:

"*Communication on projects is a strategically planned process that starts at the outset of the action and continues throughout its entire lifetime, aimed at promoting the action and its results. It requires strategic and targeted measures for communicating about (i) the action and (ii) its results to a multitude of audiences, including the media and the public, and possibly engaging in a two-way exchange.*

Dissemination is a specific term for the H2020 programme. It means to make the results itself of a project public (— by any appropriate means other than protecting or exploiting them, e.g. scientific publications). The public disclosure of the real results by any appropriate means (other than resulting from protecting or exploiting the results) to be used, including by scientific publications in any medium."

The success and efficiency of C&D activities can be seen and enhanced by further adopting exploitation strategies. This allows the uptake of TNs results.

A former study, showed that factsheets, practice abstracts and videos are the TNs most preferred dissemination materials (Mosquera-Losada et al. 2020, in press). This paper presents the different ways of communication and dissemination, related to the involvement of the end-user groups. Moreover, an analysis of 28 state-of-the-art TNs within the EURAKNOS project and 26 face-to-face interviews were carried out. This analysis highlights the used C&D strategies, ways, tools, and channels of 26 TNs, with a major focus on social media.

Material and Methods

The study was carried out in two steps: a desk-top study linked to the TN web pages, and a set of interviews and surveys. The "desk-top study" was based on an analysis performed by cross-linking information available on TN websites and C&D Plans, allowing to obtain an initial overview linked to the C&D plans and the further implementation associated to the evaluation of the 28 TNs' webpages about the approaches to communicate and disseminate the innovations the TN developed. In particular, the attention was focused on C&D tools and channels, created to reach the TNs' target audiences. In particular, the produced outputs communicated and disseminated through the different social media channels (YouTube, Twitter, LinkedIn, and Facebook) were investigated. For each channel, their key performance indicators (KPIs) as stipulated in the TN project, were considered, such as the number of followers, views, shares, visits, and downloads for digital channels, as well as the number of

participation in events for physical meetings. These numbers are proof in terms of the number of people reached but are also a good tool to get an idea of the success of the implemented activities.

Once an overall picture of TNs was acquired, a set of interviews was performed to validate and complement the results obtained in the desk-top study. The questionnaire was divided into two parts, carried out in parallel. The first part was a face-to-face questionnaire to capture the detail of C&D strategies, feedback, learnings, and reflect on best practices. The second part contained an online survey that aims to identify target groups and types of C&D material.

The face-to-face interviews were conducted between June and September 2019 by the EURAKNOS consortium members. More specifically, 26 face-to-face interviews and 17 online surveys were completed. The questionnaires and surveys considered multiple end-user categories (industry, policy-makers, students, farmers, researchers, and advisors), as well as multiple KPIs (available in the C&D plans and Google Analytics).

Results and Discussion

Based on the outcomes of the 26 interviews, we can conclude that 84% of the interviewed TNs developed a dedicated strategy for C&D, while the remaining 16% were aiming to develop it. Close to 54% of the TNs with a C&D plan made a clear distinction between C&D strategies with accompanying and detailed plans. However, around 31% of the interviewees indicated that they see no difference in terms of activities between C&D. Moreover, 6 TNs even mixed both terms in their strategy plans. For example, a TN titled a document "Communication Plan" although it was dealing with the dissemination and vice et versa. This phenomenon highlights the lack of clarity between both activities.

Regarding the use of digital channels, both the desk-top study and interviews confirmed that social networks are considered to have an essential value for the C&D of TNs. For example, 75% of TNs are using Twitter to communicate, whereas Facebook, LinkedIn, and YouTube are used by 55%, 48%, 45% of the TN, respectively. Moreover, Twitter is a communication tool that allows the quick circulation of a lot of information, such as the project announcement of events, delivery of reports, and catching up with new projects. Furthermore, certain platforms are more popular in certain EU countries than others, i.e., the popularity differs culturally. As an example, TEAGASC (an Irish state agency providing research, advisory, and education in agriculture) uses Twitter regularly to reach farmers and communicate their findings, whereas 25% of the TNs do not use Twitter at all for communication purposes. That is why it is important that a TN uses multiple social network accounts to reach widely all EU countries and different target audiences.

To engage followers through a social network and create added value for the communication of the project, a TN should have a regular (e.g. weekly) activity to reach at least 500 followers (which is a usual but informal indicator of engagement in social media) (Gräve and Greff 2018). We can link the success of a social media network to the amount of activity (posts, shares, likes), as illustrated in Figure 1, which summarizes the KPIs regarding activities that were found in the analyzed C&D plans. The more a TN creates activities through the social network, the more it gains followers and, therefore, impact on end-users. Figure 1 shows that there is a considerable difference in activity trough the different channels. This can be explained because of a virtuous cycle of sharing posts. In fact, when a post is made on Twitter or Facebook, it is possible to tag the different partners, which increases the impact of the post. In contrast, YouTube and LinkedIn accounts are often harder to find or do not even exist.

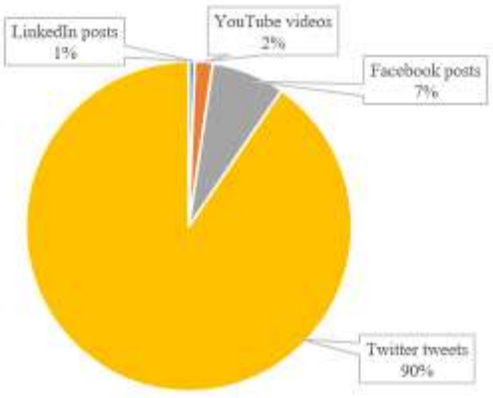


Figure 1. The total amount of posts per social network (numbers updated in March 2019).

The online survey allowed us to identify which actors were using which digital channels. More specifically, it was asked to the interviewee on which end users they focused on, on each social network. Secondly, it was asked which end users they successfully connected. For this purpose, Figure 2 represents the comparison of targeted actors for each social network. LinkedIn is excluded from the visual comparison because it only contributed to 1% of the total amount of social network posts, as can be seen in Figure 1.

Figure 2 shows that the category of researchers is more connected than foreseen by all social networks. This can be explained by the fact that TN partners and organizations are mostly researchers and universities. Students are not sufficiently involved, for example, only 4% and 5% are connected through Twitter and Facebook, respectively. However, some TNs developed educational programs and training courses directed towards students, as well as farmers/foresters and advisors. These programs are part of a key solution for the sustainability of the TNs, as it can be accessible in the long term and re-used to disseminate the knowledge and increase impact. Furthermore, during the interviews, it emerged that some TNs do not target students. Additionally, policymakers appear less reached by social networks than expected. Lastly, farmers have a good rate of connection with all types of social networks, except LinkedIn.

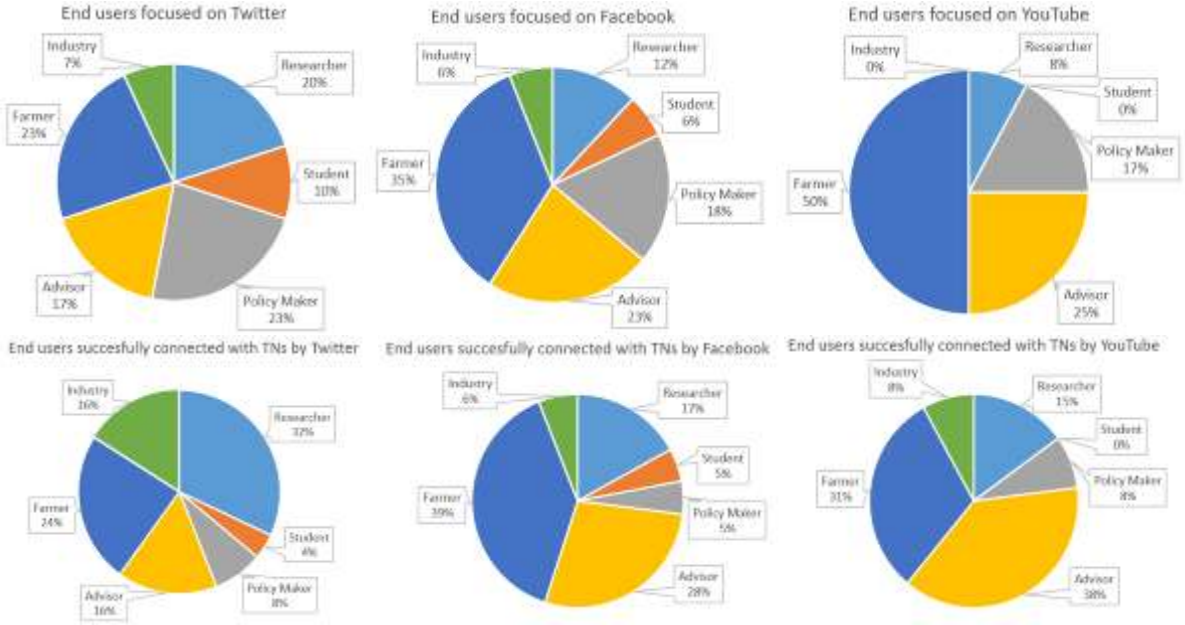


Figure 2. Type of end-users in social networks (Twitter, Facebook, and YouTube) of TNs

Since YouTube appeared to be an important tool for communication and dissemination with farmers, it was also asked the interviewees how they think to make an impactful and successful video to spread knowledge. All the TNs answered that it must be short and the message should be to the point. Moreover, 94% of the TNs think that it should contain practical content, such as a field demonstration. However, 68% believe it is also important to have scientific content. On the format, 65% would prefer an interview recording included in the video: and 65% would agree to engage professional movie makers to create these.
It should be noticed that the use and application of social media may strongly depend on other factors, such as broadband connectivity, digital skills, and social and cultural background that varies from region to region within Europe. Thus, there is a need for a more in-depth study of social media as an efficient C&D tool towards farmers, forester, and advisors.

Lastly, during the analysis, we noticed several limitations and strengths of each used approach. These are presented in Table 1.

Table 11. Overview of the limitations and strengths of the methods used for the TN analysis

Method	Limitations	Strengths
Desk-top study	Missing information	Includes facts and figures
Face-to-face interviews	TN coordinators not always available, time-consuming	Sharing of experiences of TN coordinators and partners, detailed answers
Surveys	Incomplete answers	Internal validation, time-saving, results easy to process

Conclusions

The main objective of a TN in the agricultural sector is to enhance innovation by the uptake of innovative knowledge and best practices by farmers and foresters. Delivering the right message to the right target groups for this purpose is an important challenge.
This study revealed that although all TNs employ communication and dissemination tactics, they do not always distinguish between the two. Additionally, social media networks are reactive and interactive channels that enable the spread of TN results between peers and other actors. The more active a TN is on social media, the more followers it gains. Since the added value of a social media network can be linked to the amount of activity, it is important that they are used regularly during the lifetime of these projects. Moreover, the ability to tag different partners in a social-media post increases the size of the audience reached. Lastly, during the analysis, we found that researchers and farmers are well-connected by all social networks, whereas students are currently less targeted and involved in these activities.
This study showed that social media is a powerful tool to perform C&D. However, their popularity within end-user groups depends on other factors, such as the social and cultural background. Therefore, future work will address these factors more thoroughly.

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DAIRY PRODUCTION IN WALLONIA: PROBLEMS AND PERSPECTIVES

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Abstract

Milk and on-farm dairy products represent an important share of the final value of agricultural production in Wallonia. This sector was largely transformed after the adoption of the Common Agricultural Policy, the implementation of milk quotas in 1984 and their suppression in 2015. Based on official statistical data, on a literature review about the possible futures of the Walloon dairy sector, on an exploratory workshop with regional stakeholders and on an on-line survey among representatives of the dairy sector, the research aims to identify the problems of the dairy sector and the possible solutions for the future. Being successful during several decades, the sector encountered more difficulties later on. Environmental problems appeared and the number of dairy farmers and of dairy cows sharply declined, the prices – and so the income – became unstable, especially after the liberalization of the CAP. The capacities of the dairy industry significantly increased and were oriented to the world market, while the consumption patterns largely changed. In order to overcome its difficulties, the dairy sector will have to meet new challenges: emerging consumption patterns, like vegetarianism, human health concerns, new societal expectations about the environment, animal welfare, climate change, biodiversity, landscape preservation, cultural heritage, the numeric revolution and precision agriculture…In the future, the Walloon dairy production will have to be based more significantly on grassland and less on concentrates and imported feedstuffs, with diversified cattle races, larger quantities of milk processed and sold on-farm, more quality-specific (including organic) products, and the organization of production and marketing cooperatives.

Keywords: *Dairy production, Challenges, Perspectives, Wallonia*

Introduction

Dairy production is a very important activity in Walloon agriculture. In 2018, milk and on-farm processed dairy products represented 25% of the total value of the final Walloon agricultural production. In 2016, 12.8% of Walloon farms were specialized in dairy production, while 13.9% were specialized in both milk and bovine meat production and 11.7% in both milk and general crops (DAEA, 2019). Though the territory of the region is small (16,000 square kilometers), natural conditions are largely variable and so agricultural production and cattle raising characteristics are diversified (Petel et al., 2019). Belgium being one of the six founders of the European Union, the Common Agricultural Policy (CAP) deeply influenced the evolution of its agriculture, including the dairy sector. Farm specialization was stressed; yields, inputs consumption, production, farm size and mean herd per farm increased, while the number of farmers sharply declined. These phenomena were strengthened after 1984 and the implementation of dairy quotas. For the remaining farmers, dairy production remained nevertheless profitable. However, some problems, new consumption patterns and new societal expectancies progressively appeared, linked to environmental concerns, human health, animal welfare…

The objective of this paper is so to identify the problems and perspectives to which the Walloon dairy sector is and will be faced and to explore the possible solutions which could be implemented in the future.

Material and methods

Statistical data have been collected in 2020 from official sources like the regional Direction for economic agricultural Analysis (DAEA), which manages the Walloon Farm Agricultural Data Network, and the federal Ministry dealing with statistics and economic information (Statbel), as well as from the Belgian Dairy Industry Confederation (CBL).
Concerning the more qualitative data, information was gathered through two ways (Godet, 2007).

a) Literature review
   The literature review was made at two levels
   - The general analysis of the perspectives of dairy production from the international point of view
   - The specific analysis of the situation and perspectives of the Walloon dairy sector
b) Opinions of actors and experts
   Experts and actors were interviewed through two ways:
   - An exploratory workshop was organized in December 2019. Based on the main features of the present situation of the Walloon dairy sector, the participants were asked to give their opinion on the main problems and perspectives of the sector
   - A questionnaire on-line was sent in March 2020 to participants who previously agreed to answer it. The participants were asked to give their opinion on a prepared text and to determine what were the main threats and opportunities of the bovine sector according to their experience.

Results and discussion

In 1984, when the dairy quotas were implemented, the number of dairy cows in Wallonia reached nearly 460,000 heads. In fact, many "dairy" cows were raised to produce both milk and meat. The new system, which limited the quantities of milk which could benefit from guaranteed prices, led to a greater specialization of herds and farms. The yields significantly increased and the number of cows needed to reach the maximal quantities of milk decreased consequently to 267,000 in 2000 and to a minimum of 196,000 in 2017 (- 57% compared to 1984), after the dairy products crisis on the world market. In 2018 and 2019, the number of dairy cows increased a few up to nearly 200,000 (Statbel, 2020).
Meantime, the number of dairy farms drastically declined from 19,400 in 1984 to 4,300 in 2019 (- 78%). So, the mean number of dairy cows per farm increased from 24 in 1984 to 46 in 2019 (X 2). The mean delivery to the milk processing plants also sharply increased in Belgium, from 76,000 liters per farm in 1984 to nearly 600,000 liters in 2019 (460,000 in Wallonia). Among the deliveries, the share of organic milk increases regularly and reached 2.16% in 2019 (5.9% in Wallonia). Nearly all Belgian farmers respect the quality standards and procedures under the QFL (Qualité Filière Lait – Quality Dairy Sector) which are beyond the public compulsory standards.
Till the reform of the milk and dairy products common market organization, milk production remained profitable for the dairy farmers still in activity. However, the reform (the "soft landing") which finally led to the suppression of the milk quotas made prices paid to farmers, and so the income, much more variable and unpredictable than before. The mean price for 100 liters of milk paid to the farmers (including premiums) reached 34.67 € in 2019 in Belgium.

However, it reached only 24.74 € in 2009 and 29.10 € in 2016, years when a crisis happened on the market, while a maximum of 39.44 €/ 100 l was observed in 2013 (CBL, 2020). As a result, the income per working unit, which was the most stable compared to other specializations like general crops or bovine meat, became very variable during the last years: being as low as 6,391 € in 2016, it reached 29,791 € in 2017 (X 5) for the farms belonging to the orientation "dairy" in the Walloon FADN. In such conditions, the income of dairy farmers is heavily depending on direct payments and on rural development payments. In 2016, the payments from the first pillar of the CAP represented 208% of the income per working unit in the dairy specialized farms, while the payments from the second pillar counted for 35% of the income. In other words, the income would have been negative without the financial support of the CAP (at least when a calculated cost is allocated to family labour). In 2017, the figures went down to 43 and 11%, respectively (DAEA, 2019).

Price instability and income variations, however, are not the sole problems to which dairy farmers are and will be faced. Many changes and evolutions can be expected for the forthcoming years.

Consumption patterns are changing after several years of a continuous evolution in the same direction. The purchases of fresh milk and butter by the Belgian households declined regularly during several decades (Commission filière laitière, 2019). However, this decrease stopped in 2019: the purchases of fresh milk and butter reaching 42.65 liters and 2.15 kg per capita, respectively (CBL, 2020). After a significant increase, the purchases of yoghurt began to decline slowly during the last decade and passed for the first time since many years below 10 kg per capita (9.87) in 2019. The purchases of cheese also drastically increased in the past and reached 15.90 kg per capita in 2010. Later, they declined to 14.08 kg in 2018 and were stabilized at 14.12 kg in 2019. The purchases of cream increased till 2015 (2.62 kg per capita) and slowly declined since then (2.39 in 2019). So, it is clear that the consumption patterns are evolving continuously and can lead to significant changes in the long run, to which the producers must adapt.

The consumption of dairy products is still driven by the population size and the households' available income, but new considerations appeared during the last years and can have a significant impact in the future. Human health is one of the most important. Milk and dairy products were considered as very healthy and their consumption was recommended by the nutritionists. Later on, however, nutritionists claimed that a too high consumption of animal fat could lead to heart problems, arguing that vegetal oils are healthier. Other potential health problems were evoked. Though the positive impact of the consumption of milk and dairy products on human health is still recognized by a large majority of human nutrition specialists, the recommended quantities to be consumed daily have been reduced and some consumers have indeed reduced or suppressed their use of dairy products, especially butter, cream and full fat milk. The use of large quantities of antibiotics is also a new concern for human health, as microbian resistance could appear.

Animal welfare has also become an important topic for some citizens (the welfarists). The conditions in which the dairy cows are raised and produce now matter to the consumers (Delanoue and Roguet, 2015). Animals are considered to have some rights (the "antispecist" movement considers that they have the same rights as human beings). Dairy cows must benefit from a minimal space, cannot be kept all the time inside stables… The respect of animal welfare standards, which is now compulsory for the farmers in order to get the public financial support, has consequences on production methods and costs. However, some people go even further: vegetarians do not eat meat, but accept to eat dairy and other animal products; vegetalists do not eat any animal product but accept to use animal products like wool; the "vegans" do not use any animal product at all.

The environmental concerns are also growing in importance. In the past, cattle was raised on pastures and fed with forage produced on-farm and organic manure, produced in limited quantities, was used on-farm as a fertilizer (circular economy). Today, there are many systems, even in Wallonia, but some of them are input intensive and provoke water and air pollution (nitrates, phosphates). In addition, dairy cows produce non-negligible quantities of greenhouse effect gazes (methane). Intensive systems also require high quantities of energy for the importation of feedstuffs like soybean meal or tapioca, the production of fertilizers and pesticides for feedstuffs, the exportation of dairy products overseas (far away markets are growing in importance for Belgian dairy products (CBL, 2020))…

The abovementioned factors could lead to the decline of dairy production in Wallonia. However, at the same time, the demand at the world level is significantly increasing, due to population growth and higher income in developing countries (FAO, 2009). In such conditions, the Flemish dairy farmers have decided to produce more and more intensively. What will be the farmers' choice in Wallonia? Several paths could be followed.

The implementation of new production techniques can be an opportunity for some farmers. It is the case of organic farming, which is growing in importance in Wallonia. In 2019, organic agriculture reached 11.5% of the regional agricultural area and 14% of the farms. Pastures represent three quarters of the organic area. In 2019, the number of dairy cows producing organic milk represented 9.6% of the total number of dairy cows. On the demand side, it is clear that the market is expanding. The share of organic products in fresh food in 2019 reached 3.4% in Belgium and 4.9% in Wallonia. Among these organic fresh products, milk and dairy products represented 22% of the expenses by the Belgian households in 2019, when 55% of the Belgian households bought organic milk or dairy products at least once. As a mean, each Walloon citizen spent 14.8 € for organic milk and dairy products, which is more than the expenses for organic vegetables or fruits, out of a total of 70 € per capita in 2019. In Wallonia, the share of organic products reached 6.2% for fresh milk and 5.4% for processed dairy products in 2019 (Beaudelot and Mailleux, 2020). In the future, the role of organic farming will certainly be strengthened, as the sector benefits and will benefit from specific support by the regional and European authorities (European Commission, 2020).

In order to increase its resilience and to enhance the circular economy, dairy production could be linked again to forage produced on-farm, and more specifically to permanent pastures, which represented 43% of the Walloon agricultural area in 2018. For the farms specialized in dairy production, the feed produced on-farm counted only for 5% of the total operational costs (119 € / 100 l, which corresponds to 29% of the total production costs, including family labour) in 2018, against 52% for foodstuffs bought on the market (DAEA, 2019). These figures show how dependent are the dairy farms on off-farm produced feedstuff, mainly imported from non-European countries. To rely more on permanent pastures, where they are the only possible way to use the land due to soil and climatic conditions, could reduce production costs and be favourable to the environment (permanent pastures stock the carbon dioxide and avoid soil erosion) and would valorize grass, which is not directly usable by human beings. This would also avoid land competition between food and feed. This stronger link to the territory would also be favourable to the maintenance of landscapes, the conservation of local traditions, agricultural practices, social and man – animal relations, and the development of traditional or new local products with specific characteristics (Hervieu, 2002; Ryschawi et al., 2015; Duru et al., 2017; Neumeister et al., 2018). In such a situation, the dominance of the Holstein race would weaken and more robust races, producing both milk and meat, would increase their share within the regional bovine herd (La Spina, 2017)

Product marketing is also to be considered. Most dairy farmers deliver their production to milk processing plants and are price takers, with no contact with the market and the final consumers. In order to be better informed on the demand and to keep a larger share of the

value added to the final products, farmers have an interest to invest time and capital in processing and marketing dairy products rather than to sell fresh milk as raw material (La Spina, 2016). In Wallonia, dairy farmers are often members of large cooperatives which collect and process milk, but their weight is individually weak. Being members of smaller cooperatives (like Fairebel, Coferme…), of associations including producers, processors, retailers and consumers or of producers associations (like "dairy producers associations" or " dairy producers groups"), they can hold more decision-making power and make their business more profitable. In such cases, the stress should be put on specific quality characteristics, these products being supported by the regional and European policies (origin-labelled products…). Direct sales to the consumers, on-farm or on open markets, to the restaurants…is another option, though the quantities are limited.

New technologies could improve or worsen the situation – as usual. A cost/benefit analysis must be undertaken for each particular farm. However, what is now called "precision agriculture", thanks to digital tools, could have a positive effect, both on production costs and on the environment, as its goal is to reach the minimum use of inputs in order to get a given production level (Allain et al., 2014; Hostiou et al., 2014).

Conclusion

The dairy sector in Wallonia was deeply restructured during the last decades, due to the implementation of the Common Agricultural Policy, modernization and economic development. However, new problems appeared and new expectations emerged from the consumers and citizens. Under the pressure of both internal and external factors, dairy farmers must adapt continuously their ways of production and management of the farm, taking more into account non financial aspects like the preservation of the environment, human health, animal welfare or traditional ways of life. Due to the still relatively small size of its farms and to the relative availability of permanent pastures, Wallonia could try to link more and more dairy production and on-farm forage production and put the stress on qualitative aspects (specific characteristics of the products, environment-friendly ways of production, relations between farmers and consumers/citizens…) and on farmers associations and involvement in processing and marketing in order to have a better knowledge of the demand and keep a larger share of the added value.

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SMALL FARMS' AND AGRICULTURAL HOUSEHOLDS' LABOR PERSPECTIVE, CHALLENGES AND KEY INFLUENCE FACTORS IN ROMANIA

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Abstract

The Romanian agriculture has undergone a long and consistent structural adjustment starting from the EU pre-accession period in the early 2000's continuing with the EU integration period along the first two programming and budgeting periods, from 2007 until the present. One important feature of the Romanian rural economy present along the transition period was the dominance of primary sector, a reality that is still valid nowadays. Inside the sector, one important characteristic is the labour coupled with the social dimension of the agricultural activity and the rural life in general. Most research and analysis references indicate a dual agriculture originating basically in the structure of property and labour input leading, or rather preserving, a highly efficient and modern agriculture with large and very large farms and a second component less competitive, neighbouring the subsistence level and largely populated throughout the labour inputs and structured in small and very small farms and agricultural households. The present paper analyses the labour related evolution of this second category, captures the recent changes and elaborates on the perspective and the challenges driven by the potential shifts of the key influence factors identified as: education - achieved level and system's readiness to provide for the specific sectoral needs, demographics and age structure layered from farm's head to family members, migration pressure from the agri-industry component or other sectors and territorial development gaps. framework factors (land cadastre, property transfers, social support measures, support programs). The projections focus on changes and impact highlighting intervention directions leading to potential policy changes.

Keywords: *Agricultural labour, challenges, change factors, small farms, Romania.*

Introduction

Employment in agriculture as share of total employment worldwide dropped from little under 44% in 1991 to 27% in 2019 (ILOSTAT, 2020). This drop is usually a sign of competitiveness increase in developed countries, yet one-third a global level is not originating in modernisation. Further linked indicators drop accordingly as the labour is not only a factor in this case but also a main or single income source for the agricultural small households, particularly in the developing countries. Meanwhile, in Europe, the regular agriculture labour force declined by 9.5 million persons between 2005 and 2016, a reduction representing almost one third (-31.7%).

According to Eurostat the largest employer in Europe back in 2016 was the agricultural sector with almost ten million people occupied (9.7 million) still they represented less than 5% (4.2%) of the total European employment. This large crowd of people was profiled as relatively old as only 11% of the farm managers had less than 40 years and male-dominated as less than one third were females (28%) in 2016. The share of farmers of 65 years of age or more are particularly high in Portugal (51.9 %), Cyprus (44.6 %), Romania (44.3 %) and Italy (40.9 %). Most farm managers in the EU have only practical experience in terms of professional training, this category accounting for 68.3% of the total against 9.1% farm managers with full agricultural training, and 22,6% with basic agricultural training. The extremes of this amplitude were recorded in Romania and Greece with only 0,4 % and respectively 0.6% of farm managers having full agricultural training and a majority of 96.7% and, respectively, 93.2% having only practical experience opposed to the situation of farm managers with full agricultural training in Luxembourg (52.5%), Czech Republic (38.7%) or France (34.9%) (Eurostat,2020). From the 10.5 million farms in the EU in 2016, the vast majority (95.2%) classified as family farms. Based on the FAO definition, the term 'family farm' is used to refer to any farm under family management where 50% or more of the regular agricultural labour force was provided by family workers. Most farms in the EU (93%) in 2016 were farms with only family workers. Across all the farms in the EU-28, family farms used 81.4 % of the regular agricultural labour force and farmed a little less than 2/3 of the total agricultural land area. Family farms covered actively 108 million hectares of land in 2016, which represented 62.3%) of the EU-28's utilised agricultural area. In terms of animal production, the family farms reared almost 2/3 (62.5%) of all livestock and produced almost 60% of the agricultural output in 2016 (Eurostat,2020). Family farming dominates EU farming and is likely to continue to do so for the foreseeable future (Davidova and Thomson, 2014). There were 9.9 million family farms in the EU-28 in 2016, accounting for at least 90% of all farms in 18 of the Member States of which over one third (3.4 million) were located in Romania and a second third was located cumulatively in Poland (1.4 million), Italy (1.1 million) and Spain (0.8 million) - (Eurostat,2020). A review of the major studies and project reports on agriculture and rural markets where the results from the surveyed studies have covered several aspects, such as the time allocation decisions of individuals, supply of labour on- and off- the farm, mobility decisions, inter-sectoral labour adjustments, pluri-activity, structural change, the differences in farm structure and the dependence on hired labour, the differences in labour productivity and others indicate heterogeneous conditions across countries. In the case of farm household members, the results in the literature suggest that labour allocation between on-farm tasks and off-farm employment is quite elastic and appears to be heavily determined by the individual's personal characteristics, the characteristics of the household's farm and conditions in the macroeconomic environment (Tocco et al., 2012).

Examining the impact of the Common Agricultural Policy (CAP) subsidies on labour allocation in four EU member states, focusing on the decision of individuals to exit the farm sector, Tocco et al. (2016) find that the total subsidies at the regional level are found to be negatively associated with the out-farm migration of agricultural workers in Hungary and Poland. This finding indicates that the CAP would seem to preserve jobs in the farm sector, and therefore implies farm survival being effective in transferring income to farmers.

A number of authors have early indications about the importance of the migrant workers, particularly seasonal workers moving from the Central, Eastern and South Eastern Europe towards the old EU member states. Donaldson (2015) quotes the French farmer Nicolas Duntze, also a founding member of the Confédération Paysanne, a union for peasant farmers - "Without migrants, European agriculture will not survive". Although assessed as important particularly for the high labour-intensive crops, the seasonal migration of agricultural labour seen more clearly during the 2020 SARS-COV-2 pandemic comes packed with specific

issues, namely exploitation and abuses. Danielsson et al. (2020) estimate the extent of labour exploitation in European agriculture as huge. The European Federation of Food, Agriculture and Tourism Trade Unions estimates that some four million agricultural workers, many of them migrant workers, operate in conditions of illegal employment, precarious working and exploitation, whether as seasonal workers, day labourers or otherwise insecure. The authors advocate that EU funding to employers with unacceptable working conditions and working environments should be denied, considering that agriculture receives a large part of the EU budget as 38%. The analysis aim to focus on the labour related evolution capturing the recent changes and elaborating on the perspective and the challenges driven by the potential shifts of the key influence factors.

Material and Methods

The paper relies on desk research carried out over the secondary data collected by formal public structures and used for reporting at national level within the Annual Statistics, in specialised approaches, such as the Agricultural Censuses; also the national datasets and databases are mounted based on harmonised and agreed standards in Eurostat. Currently the secondary data at these levels are consistent and reliable, however certain indicators are not mirrored at both levels (EU and National). The choice of scale at regional level aims to avoid the biasing or levelling by regional differences, production specialisation and profile or impact of cultural model. The selected region presents a mix of unique features with regard to the rural economy and more particularly the agriculture as dominant all while being under an important migration pressure, bot emigration and immigration. This last condition was considered of high relevance for the analysis of the agricultural labour paralleling the last two EU programming periods and funding support. Considering the formulated conditions, the South West Oltenia Region (RO41) at NUTS 2 scale was selected. The particular interest for the extra pressure conditions, eventually favouring or preventing the labour changes, are highly important for the defined territory. The selected observations are used to understand the agricultural anthropic environment and analysed for the potential influence factors and drivers. The findings are structured and presented in interrelation to support the challenges formulation and perspective.

Results and Discussion

Under the given conditions and the highlighted characteristics of the Romanian agriculture, as presented in the introductory part, the paper targets the challenges and the key influence factors of the agricultural labour in small farms and agricultural households. The methodologic choice of eliminating any bias by average among different regions allows an educated choice introducing supplementary pressure factors. In this respect, the choice adds the particular migration pressure (emigration and seasonal migration) at the regional scale, articulated with a consistent share of favourability for agriculture in terms of geographic conditions, market and general logistic, soil quality, production diversity and specialisation.
Southern and Western EU countries (Spain, Italy, France, Germany, UK) are receiving large number of seasonal agriculture workers originating from the region adding to the permanent emigration, although the statistics fail to capture accurate figures. Also, the rural youth is split in orientation towards two main inland regions - the national capital and the western most developed city. Also, one particular feature of the region is represented by the strong industrial profile until the 90's consisting of a large number of industries from chemical to mechanical, from automotive, train locomotives, to airplanes. This aspect is relevant considering the industrial collapse during the transition period (1990-2000) and the use of

agriculture and agricultural households as social buffer and safety net for all the unemployed people, formerly active in industry.

According to the OECD methodology (OECD, 2007) the selected region qualifies as predominantly rural, with over 50% rural population (51.91%) or 1,156,185 people, determining a general density of 76.2 inhabitants/km^2. The region's rural population represents 12% of the total rural population, as one of the eight regions of Romania, an average level and balanced distribution. Administratively, the region is structured in 408 municipalities comprising 2,070 villages spread over five counties (NUTS3 level).

The agriculture's gross value added records numerous hiccups and lacks a trend over the past two decades, partially due to the Romania's accession to EU and the structural adjustments performed to adapt to the new open market pressure, partially as result of the population drain from the region, as over 300.000 people (equivalent of one third form the rural population) moved away only during the period 2005-2016 affecting importantly the reporting base.

At regional level the total regular labour force amounts to 1,038,650 people out of which 1,032,590 are represented by family labour force (sole holders and family members). At sectoral level, these figures represent 33.5% as share of active population involved in farming activities (2017). Interestingly enough, the share of female sole holders working on farm reaches 53.5% while in terms of the share of family labour almost entire volume of persons involved is represented by females (99.4%).

The age structure of the farm managers is relatively well synchronised with the national average distribution with 3% under 35 years, almost 29% between 35-54 years while 68% have 55 years and more in 2016. A more balanced situation is recorded for the female heads of agricultural holdings without legal status, where 21% have less than 45 years, 30% have 45-64 years and 46% have 65 years or more. However, the female farm heads represented only 35% from the total in 2010 (MADR, 2010). While the number of sole holders working on the farm decreases by almost 10% from 2005 to 2013, the number of female sole holders increases by 5% promising an interesting decline compensation.

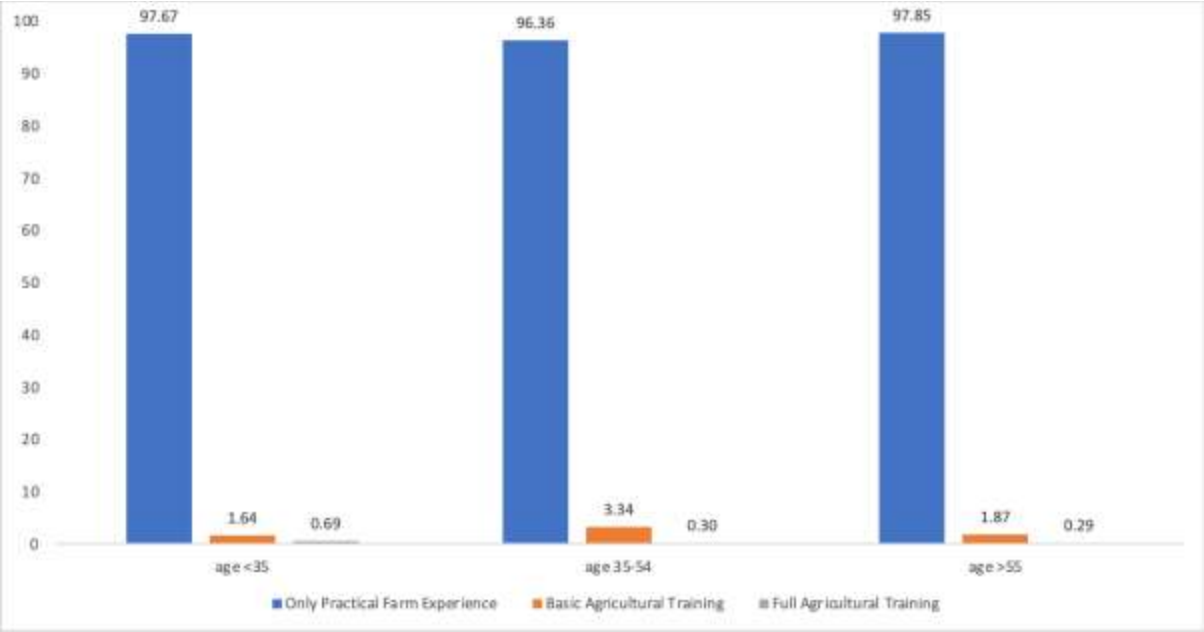


Figure 1. Level of education of farm heads by age groups in RO41, in 2010.
Source: Processed data from Farm Structure Survey, Romania, 2010.

The level of education and professional training of the farm heads in the region is highly unbalanced, mirroring the situation at the national level, with systematically over 95% of farm

heads having only practical experience, while the full agricultural training barely overpasses 0.5% in the case of the farm heads under 35 years (Figure 1).
The number of farms and agricultural households with other gainful activities has increased in the case of sole holders from 18,260 farms in 2005 to 84,230 farms in 2013. This increase in number was accompanied by an enlargement of Utilised Agricultural Area with 1,109,310 ha and an output of 1,811,371,910 Standard Output.
The depicted situation introduced briefly above allows the compilation of observations in the quest for the key influence factors and projecting the challenges for the agricultural labour originating in small family farms and agricultural households.
The prime factor appears to originate in the demographics, and it is represented by the age of the farm head and the family labour aiding at farm/household level, or better targeted, the very unbalanced shares of age categories and potential natural replacement. This distribution can act as highly un-motivating vector for the youth from the region determining shifts in professional orientation and threatening the continuity of small farming to a certain extent.
A second factor is represented by the education and professional training levels of the agricultural labour seriously challenged by the constantly increasing demand for specialisation and modernisation knowledge requirements.
The third factor is represented by a structural issue consisting of a dual agriculture with a very high number of small farms and agricultural households, low competitiveness and rather subsistence-oriented coexisting with large-scale commercial farms performing well, rather agricultural industry operations.
A fourth factor is represented by the migration pressure supported by the regional development level differences, employment opportunities from within the sector inside the region, from the neighbouring regions, or from the industry, services and constructions. The emigration trend continues to drain the middle-aged rural population and couples with the seasonal workforce migration to EU.
The fifth identified factor is a compilation of framework factors consisting of land cadastre (presence and operation), land market and property transfers, social support measures and support programs.
These five key factors are challenging the rural labour of the region, particularly the people from the agricultural households and small farms and the young generation with more elasticity in education and professional reorientation, further challenging the sector's labour availability.

Conclusions

Coping with the future uncertainties in terms of agricultural labour and the challenges induced by the identified key factors could start with the unhappily coupled factors age-education. The changes can be supported by designing and implementing more youth-targeted support instruments with particular emphasis for the rural youngsters. Current EU and national funded programmes don't discriminate but equally lack the specific focus introduced earlier. Youth targeted policy instruments supporting them on medium term can temperate the replacement generation drain from the region.
The second component could be addressed by institutionalising an education and training framework for agricultural professionals. Presently there is no formal education or training programs for agriculture and related activities at national, regional or local level, of any kind. The attempts implemented via different projects are far from reaching a critical mass and lack consistency and presence due to the dependence on the project life duration. A coherent and systematic implementation of the new educational framework could be complemented by the

introduction of adult training and education programmes targeting long-term improvement and systemic consolidation.

Since negotiating and implementing a new component specific to the small family farms and agricultural households, flavoured more like social-agriculture, although of high relevance for certain Central, Eastern, South Eastern and Mediterranean EU member states, is highly unlikely to happen within the frame of the Common Agricultural Policy, the remaining option could incorporate the specific support within a national level improved social policy including the agriculture-dependent families.

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ANALYSIS AND FORECASTING OF VEGETABLE PRICES IN SERBIA[10]

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Abstract

The paper presents the analysis and forecasting of prices for seven vegetable crops: potato, bean, tomato, pepper, onion, cabbage and watermelon. The absolute vegetable prices were analyzed for the period 2002-2017, staring thus from the year the euro was introduced. Conversion of the prices into euro was carried out according to the average annual exchange rate of euro, based on the data of the National Bank of Serbia. The analysis of the real prices, which are price parities of certain vegetable crops in relation to bread grain (wheat), was conducted for the whole period 1994-2017. The aim of this analysis was to formulate the relative changes of the economic position of certain vegetable crops in relation to wheat, which is the most important cereal cultivated in our conditions. Forecasting of the prices for certain vegetable crops was made for a five-year period 2018-2022. ARIMA models were used for the forecast, indicating that all average vegetable prices analyzed for the period 2002-2017 will increase significantly in 2022. On the other hand, the average price parities of the vegetables in relation to wheat as analyzed for the period 1994-2017 will not have the same trends in the forecast period as the absolute prices. The bean price parity will improve from 9.1 to 12.3; tomato from 1.9 to 3.5; pepper from 2.3 to 3.

Key words: *vegetables, price, forecast, Serbia*

Introduction

Analysis and forecasting of the prices of vegetable crops was carried out for the period 1994-2017. Since it was necessary to take into account the effect of inflation in the entire analyzed period, the analysis and forecasting of the economic parameters was directed in two ways: analysis and forecasting of the absolute vegetable prices and analysis and forecasting of relative prices, i.e. price parities of certain vegetable crops in relation to the price of bread grain, i.e. wheat.

Due to (un)availability of data, the analysis and forecasting of economic characteristics of the vegetables were performed for seven out of ten studied vegetable crops: potato, bean, tomato, pepper, onion, cabbage and watermelon. In the analysis of the absolute prices, the average annual prices of the vegetables were converted into euro per ton, in order to be able to compare the prices with foreign countries and reduce the effect of domestic inflation. The absolute vegetable prices were analyzed for the period 2002-2017, staring thus from the year the euro was introduced. The forecasting of the vegetable prices was made for a five-year period (2018-2022).

The authors of this study have already published a number of papers dealing with analysis and forecasting of vegetable prices (Ivanišević et al. 2016; Novković, Mutavdžić 2016; Mihajlović

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et al. 2019; Novković et al. 2019), as well as vegetable price parities (Novković et al. 2018; Novković et al. 2019a).

Material and Methods

Methods used for the analysis of absolute and relative prices (price parties) of the vegetables are the methods of descriptive statistics. The analysis of the relative prices, i.e. price parities of certain vegetable crops in relation to the price of bread grain (wheat), was conducted for the period 1994-2017. The absolute vegetable prices were analyzed for the period 2002-2017, i.e. from the introduction of the euro.

In an attempt to forecast the studied price changes, the method of time series analysis was used and based on the time series analysis we applied ARIMA models. The time series analysis was conducted using the price characteristics of the vegetables in the stated period, while the forecast refers to the following five-year period 2018-2022.

The series of the observed phenomena in this paper are acquired, or formed, based on the statistical publications of the Statistical Office of the Republic of Serbia. The statistical software Statistica 10, Eviews 3.1 and SPSS were used for the analysis of the collected data.

Results and Discussion

The average price of potato in Vojvodina in the period 2002-2017 amounted to 183.3 EUR/t. The price ranged from 84.5 EUR/t in 2005 to 249 EUR/t in 2013. The coefficient of variation was relatively high, amounting to 27%. The average annual price of potato had a rather pronounced increasing trend, at an average annual rate of 3.83%. The relatively high growth rate of the potato price is an indicator of the absolute improvement of its market position.

The model for analysis and forecasting shows that the potato price in a current year is significantly affected by random processes from the previous two periods.

Based on the estimated model, the potato prices were forecast showing that in the following five years the potato price will oscillate over the years, which was also the case in the analyzed period.

The relative price of potato in relation to wheat in the analyzed period from 1994 to 2017 averaged 1.42. It means that 1 kilogram of potato was worth 1.42 kilograms of wheat. The parity ranged from 0.93 in 2005 to 2.7 in 2000. The relative variation of the potato price corresponds to the absolute variation, which is indicated by almost the same coefficient of variation of 26.6%. The relative price of potato does not show an increasing trend, as was the case with the absolute price, but a slight decline at an average annual rate of -0.89%. This means that, despite the absolute increase in the potato price, its relative economic (price) position in relation to bread grain slightly deteriorated in the observed period.

The forecasting model shows that the potato/wheat price parity was statistically significantly affected by the value of this parity from the previous two years.

The forecast values of the potato parity show that the forecast period will be also characterized by oscillations. The parity will range from 1.41 to 1.45 kg of potato per 1 kg of wheat. At the end of the forecast period (2022) it is expected that about 1.41 kg of potato will be needed for 1 kg of wheat.

The average annual price of bean was 1,333.6 EUR/t and ranged from 948.4 EUR/t in 2004 to 2,213.2 EUR/t in 2014. The coefficient of variation was moderately high at 28.5%. The average annual growth rate of the bean price was 1.33%. This means that bean showed a trend of slight improvement of its absolute economic (price) position on the market.

It is expected that in the forecast period, the bean price will alternately fall and rise over the years.

The average annual parity of the bean price in relation to wheat was 9.14. The parity varied in the range from 4.43 in 1996 to 14.54 in 2014. The coefficient of variation was 27.65%. The relative price of bean showed an increasing trend, as was the case also with the absolute price, at an average annual rate of 2.19%. This means that, apart from the absolute increase in the bean price, its relative economic (price) position in relation to wheat also improved.

In the following period, the ratio of bean and wheat prices will oscillate over the years: it will show an increasing trend until the middle year of the forecast period, followed by a decrease in the parity value until the end of the forecast period. The parity oscillations range from 10.80 to 13.63. In the last year of the forecast, the parity will be 12.31, which is significantly above the average parity in the analyzed period.

The average annual price of tomato in the analyzed period was 298.2 EUR/t. The price changed in the interval from 106.6 EUR/t in the initial year of the analyzed period (2002) to 564.9 EUR/t in 2014. The variation coefficient of the average annual price of tomato is extremely high and amounted to 44.6%. The average annual rate of change in the tomato price was the highest compared to other analyzed vegetable crops and amounted to 9.1%. This means that tomato had the most pronounced trend of price increase of all analyzed vegetable crops, i.e. it showed the strongest trend of improvement of the price conditions for production.

The forecast tomato price in the following five-year period showed an increasing trend.

The average annual parity of the tomato price in relation to wheat in the analyzed period in Vojvodina was 1.89. The parity varied in the interval from 0.63 in 1996 to 3.71 in 2014. The relative variation of the tomato price corresponds to the absolute variation, and the coefficient of variation was extremely high: 47.8%. The relative price of tomato also showed a growing trend at an average annual rate of 3.17%. This means that not only the absolute price position of tomato but also its relative economic (price) position in relation to wheat improved, but to a much lesser extent.

The values of tomato/wheat parity in the forecast period will increase over the years, ranging from 3.13 to 3.47. At the end of the forecast period (2022) 1 kg of tomatoes will have a value of 3.47 kg of wheat.

The average annual price of pepper was 310.5 EUR/t. The price varied in the range from 188.3 EUR/t in 2006 to 447.8 EUR/t in the last year of the analyzed period. The variation coefficient of the average annual price of pepper was high and amounted to 31.3%. The average annual rate of change in the pepper price was very high and amounted to 5.87%. This means that pepper also showed a significant trend of absolute improvement of its economic position.

The estimated model provided the values of the pepper price for the following five years, showing that the pepper price is expected to increase continuously over the years until the end of the forecast period.

The average annual parity of the pepper price in relation to wheat was 2.29. The parity varied in the range from 1.31 in 1997 to 3.29 in 2000. The relative variation of the pepper price is slightly more moderate than the absolute one. The coefficient of variation was moderately high: 26.7%. The relative price of pepper in relation to wheat also showed a growing trend at an average annual rate of 1.13%, which is almost two times lower than the rate of the absolute price increase. This means that, in addition to the absolute price position of pepper, its relative economic (price) position also improved, but to a much lesser extent.

The price parity of pepper/wheat will have a decreasing trend in the following period and at the end of the forecast period 1 kg of peppers will be worth 3.04 kg of wheat, which is 25% higher parity compared to the average in the analyzed period.

The average annual price of onion was 178.2 EUR/t, ranging from 119 EUR/t in 2004 to 270.7 EUR/t in 2011. The variation coefficient of the average annual price of onion was moderately high (but the lowest in relation to other analyzed vegetables) and amounted to

22%. The average annual rate of change in the onion price was positive and amounted to 1.63%. This means that the price of onion had a slightly increasing trend in the analyzed period, i.e. its economic (price) conditions for production showed a trend of slight improvement.

The estimated model indicated the movement of the onion price in the period from 2018-22. The forecast values showed that the onion price will constantly increase over the years during the forecast period.

The average annual price parity of onion in relation to wheat in the observed period was 1.52. The parity varied in the interval from 0.87 in 2012 to 4.17 in the initial year (1994) of the analyzed period. The relative variation of the onion price does not correspond to the absolute variation, as the coefficient of variation is extremely high, 42.7%, which is almost twice as high as the variation in the absolute price. The price parity shows a decreasing tend at an average annual rate of -5.45%, which is the most pronounced downward trend of all analyzed vegetable crops. This means that despite a slight absolute price improvement in the position of onion, its relative economic (price) position in relation to wheat significantly deteriorated.

In the forecast period, the onion price in relation to wheat is expected to oscillate. The parity value is expected to decline in the first three years of the forecast period, followed by increase in the last two years.

The average annual price of cabbage was 158.8 EUR/t. The price varied in the range from 80.5 EUR/t in 2004 to 212.5 EUR/t in 2007. The variation coefficient of the average annual price of cabbage was moderately high and amounted to 22.3%. The average annual rate of change in the cabbage price was slightly positive and amounted to 1.48%. This means that cabbage also had a trend of slight absolute improvement of its economic (price) position.

Based on the estimated model, the movements of the cabbage price were forecast for the following five-year period, showing that the cabbage price will oscillate over the years, alternately decreasing and increasing.

The average annual price parity of cabbage in relation to wheat was 1.39. The parity varied in the range from 0.84 in 2013, to 2.89 in 1994. The relative variation of the cabbage price was far more pronounced than the absolute one. The coefficient of variation was extremely high: 39.9%. The relative price of cabbage in relation to wheat showed a declining trend at an average annual rate of -3.54%.

In the following period, the value of cabbage/wheat parity will have an increasing trend in the first three years, while in the fourth and fifth year there will be a modest decline in the parity value, so that at the end of the forecast period the value of the parity will be 1.4, which is practically at the average level in the analyzed period.

The average annual price of watermelon was 118.1 EUR/t. The price of watermelon varied in the range from 68.4 EUR/t in 2004 to 213 EUR/t in 2010. The variation coefficient of the average annual price of watermelon was moderately high amounting to 31.1%. The average annual rate of change in the watermelon price was moderately high and amounted to 2.84%. This means that watermelon also showed a pronounced trend of absolute improvement of its economic position.

The forecast values indicate that in the initial years the price of watermelon will have a declining trend, as it will decrease in the first three years of the forecast period, while in the last two years of the forecast period the watermelon price is expected to increase.

The average annual parity of the watermelon price in relation to wheat was 1.09. The parity varied in the interval from 0.63 in 2002 to 2.17 in the first year of the analyzed period (1994). The relative variation of the watermelon price was far more pronounced than the absolute one. The coefficient of variation was extremely high: 41.7%. The relative price of watermelon in relation to wheat showed a declining trend at an average annual rate of -4.35%.

This means (as was also the case with onion and cabbage) that despite the slight absolute price improvement of watermelon, its relative economic (price) position in relation to wheat significantly deteriorated.

Oscillations in the price parity, which were characteristic of the analyzed period, are expected also in the forecast period. The maximum forecast value of the parity is expected in the first year of the forecast period, which will be followed by a decrease in the parity value in the following two years, and an increase in the value in the last two years.

Conclusions

All average prices of the vegetables analyzed for the period 2002-17 will significantly increase in 2022, in the following way: potato from 183 to 195 EUR/t; bean from 1.333 to 1.492 EUR/t; tomato from 298 to 517 EUR/t; pepper from 310 to 530 EUR/t; onion from 178 to 198 EUR/t; cabbage from 159 to 170 EUR/t and watermelon from 118 to 146 EUR/t.

On the other hand, the average price parities of the vegetables in relation to wheat analyzed for the period 1994-2017 will not have the same trends in the forecast period as the absolute prices. The price parity of bean will improve from 9.1 to 12.3; tomato from 1.9 to 3.5; pepper from 2.3 to 3. The parities of potato (1.4) and cabbage (1.4) will practically remain unchanged, while the parities of onion and watermelon will deteriorate from 1.5 to 1.2 and from 1.1 to 0.8, respectively.

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IMPACTS OF COVID-19 ON FOOD SECURITY AND FOOD SYSTEM SUSTAINABILITY

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Abstract

The COVID-19 pandemic has taken a heavy toll on the population and economies. It also undermined food security and food system sustainability. In this context, this review paper analyses the outbreak impacts on the different dimensions of food security (viz. availability, access, utilisation, stability) and food system sustainability (viz. environmental, economic, social), and explores possible solutions to mitigate its negative consequences. The pandemic has affected food security both directly, leading to a decrease in food production and availability, and indirectly, as lockdown, social distancing, movement restrictions and other containment measures taken by local and national governments have undermined people's ability, especially the most vulnerable, to access food (cf. food prices) and to have a healthy and diverse diet (cf. food utilisation). The impacts of COVID-19 on the stability dimension of food security will depend on the duration of the emergency. Furthermore, the outbreak had severe consequences on the overall sustainability of the food systems (cf. production, processing, distribution, consumption); disruptions of food systems caused by COVID-19 affect not only the food system functioning but also its performance and sustainability. However, food-related impacts of the pandemic vary not only from a country to another – depending, among others, on the epidemiological situation – but also among socio-economic groups. Indeed, it seems that COVID-19 is particularly affecting developing countries, whose food systems were already under strain, and poor and vulnerable households, which already suffered from food insecurity and malnutrition. Of particular concern are the impacts on children, women and indigenous communities. The COVID-19 pandemic highlighted the unsustainability of the current food systems. Therefore, recovery plans should include actions to foster transition towards a sustainable and resilient food system. Only a sustainable system could withstand severe shocks such as COVID-19 and ensure food security under similar stressful conditions.

Keywords*: COVID-19, coronavirus, food security, sustainable food systems, social vulnerability.*

Introduction

On 31 December 2019, the country office of the World Health Organisation (WHO) in the People's Republic of China picked up a media statement by the Wuhan Municipal Health Commission on cases of 'viral pneumonia' in Wuhan, People's Republic of China (WHO, 2020). On 10 January 2020, Chinese authorities determined that the outbreak was caused by a novel coronavirus (SARS-CoV-2), subsequently named "COVID-19" (WHO 2020). The exact origin of the virus is currently under investigation; however it is likely to be originated due to natural selection in an animal host before zoonotic transfer or natural selection in humans following zoonotic transfer (Andersen et al., 2020). The new virus has had a major impact on health across the world: as of August 28, 2020, more than 827,246 people have died from COVID-19 and more than 24 million cases have been confirmed (WHO, 2020). In

order to contain the rapid spread of the virus and reduce the infection rate, regional, national and local governments have implemented various forms of lockdown restrictions and travel bans (Bonaccorsi et al., 2020; Devereux et al., 2020). International efforts to control the virus have taken a heavy toll on the population and economies, and have affected several production sectors, value chains, and international trade (Bonaccorsi et al., 2020; Nicola et al., 2020). Global GDP has been estimated to decline by up to 13%, international trade has sharply declined, and countless people have lost their job and livelihood (ILO, 2020; OECD, 2020). COVID-19 outbreak has inevitably caused economic shocks and social costs that have affected food security and the functioning of agricultural and food systems worldwide (Stephens et al., 2020). In order to understand the impact of COVID-19 on food security and food system sustainability, we will use the standard conceptual framework of food security, which encompasses the four traditional dimensions (i.e. availability, access, utilisation, stability). For people to be food secure, food must be both consistently available and accessible in sufficient quantities and diversity and households must be able to utilize (store, cook, prepare and share) the food in a way that has a positive nutritional impact; these three dimensions should also be stable over time (FAO, 2008; Global Network Against Food Crises, 2020). We will use the concept of "sustainable food system" (SFS) suggested by the High Level Panel of Experts on Food Security and Nutrition (HLPE, 2014): *a food system is considered sustainable if it delivers food security and nutrition for all in such a way that the economic, social and environmental bases to generate food security and nutrition for future generations are not compromised*. Using these definitions, we can trace the link between the direct and indirect effects of COVID-19 and the rise of food insecurity and disruption of food systems observed worldwide.

Materials and Methods

The paper draws upon secondary data of scholarly literature as well as data from grey literature and databases. A search was performed on 27 July 2020 using Elsevier - Scopus database (Table 1). Additionally, 6 papers (Capone et al., 2014; Hobbs, 2020; Jones et al., 2013; Patrick et al., 2020; Petetin, 2020; Seleiman et al., 2020) have been retrieved from other sources namely Bioscience Journal, AAP News and Journals Gateway, Researchgate, the Canadian Journal of Agricultural Economics and Cambridge Core. The search included all the documents that were indexed by that date, without defining any time range or excluding any document based on publication date. No geographical restrictions were posed; however, scholarly literature is more focused on Western and Southern Europe, Northern Africa, and Northern America regions as well as China. The initial search on COVID-19 impact on food security and food system sustainability was carried out using the search string *"COVID-19" AND ("food security OR "food system sustainability")*. The search yielded more results when searching for *"COVID-19" AND "food security"* than when combining the three items together or searching for *"COVID-19" AND "food system sustainability"*. The latter keyword was rarely used, which is a clear indicator of the research gap regarding this topic.

Table 1. Summary of literature searches carried out on Scopus.

Search Theme	Search String	Number of Records Identified through the Search	Number of Selected Records	References of Selected Records
COVID-19 impact on food security	"COVID-19" AND ("food security" OR "food insecurity" OR "malnutrition")	183	11	Ahmed et al. (2020); Akter (2020); Béné (2020); Devereux et al. (2020); Fleetwood (2020); Galanakis (2020); Heck et al. (2020); Jayawardena & Misra (2020); Pérez-Escamilla et al. (2020); Rippin et al. (2020); Savary et al. (2020)
COVID-19 impact on food systems	"COVID-19" AND "food system" AND ("sustainability" OR "sustainable" OR "resilience" OR "resilient")	24	7	Ahmed et al. (2020); Altieri & Nicholls (2020); Béné (2020); Galanakis (2020); Heck et al. (2020); Rippin et al. (2020); Savary et al. (2020)

Grey sources include data from international organizations and NGOs such as the Food and Agriculture Organisation of the United Nations (FAO), WHO, World Food Programme (WFP), Global Network Against Food Crises, Food Security Information Network (FSIN), International Labour Organisation (ILO), United Nations Children's Fund (UNICEF), Save the Children Italy, ACCEL Africa (Accelerating action for the elimination of child labour in supply chains in Africa) project, European Food Banks Federation, The Global Foodbanking Network, United Nations Sustainable Development Group, United Nations Environment Program (UNEP). They also encompass editorials, articles or news from the media.

Results and Discussion

It is important to notice that food-related impacts of the pandemic vary not only from a country to another – depending, among others, on the epidemiological situation – but also among socio-economic groups. However, since COVID-19 has affected all the processes that connect food production to final consumer, it is possible to trace some common consequences for countries and people affected by the pandemic.

Food availability has not been compromised to catastrophic levels. Agriculture sectors have been typically exempted from lockdown restrictions, due to governments interest in ensuring continuity to food production (Devereux et al., 2020). However, food production has generally declined, and all stages of production (farm management practices, access to on farm-input, labour schedules, food transport, supply chains and distribution, etc.) have been disrupted (Ahmed et al., 2020; Devereux et al., 2020; Global Network Against Food Crises, 2020; Pu & Zhong, 2020; United Nations Secretary General & United Nations Sustainable

Development Group, 2020). Several factors are involved: labour restriction due to quarantine measures and loss of workforce from COVID-19 deaths and illnesses (Stephens et al., 2020); shortage of seasonal, immigrant or informal labourers due to border restriction and lockdown measures, increase in public transport costs or fear of exposure to virus (Altieri & Nicholls, 2020; Béné, 2020; Hobbs, 2020; Pu & Zhong, 2020; UN SG & UNSDG, 2020); shortage of productive inputs (seeds, fertilizers, pesticides, machinery, etc.) due to international trade restrictions and lockdown measures (Devereux et al., 2020; Seleiman et al., 2020). Movement restrictions have also increased levels of food loss; the lack of manpower available for harvesting, processing and distributing food (Altieri & Nicholls, 2020; Fleetwood, 2020; Stephens et al., 2020), the inability to transport products from the fields to points of distribution and falling consumer demand have meant the loss of millions of tons of fresh and perishable products (i.e. fresh vegetables, fruit, milk, livestock products) (Pérez-Escamilla et al., 2020; UN SG & UNSDG, 2020).

Regarding access to food, physical barriers to access were reported. Lockdown measures have forced the closure of schools, recreational places and many shops. Those selling essentials, such as supermarkets or pharmacies, needed to ensure entry quotas and social distancing inside and outside the shops (Lazzerini & Putoto, 2020). These entry-barriers undermined the most vulnerable people (i.e. pregnant women, elderly) to even enter the supermarkets (i.e. due to extremely long line-ups to get into supermarkets during bad weather conditions, fatigue, etc.) (Devereux et al., 2020; Rippin et al., 2020). This situation was exacerbated by the closure of open-air markets, street vendors and other informal markets, which led to formal outlets being the only food source available. It also led to e-shopping becoming more and more common; takeaway and home delivery have become an alternative to closed restaurants and long queues (Bakalis et al., 2020; Petetin, 2020). Panic buying and hoarding behaviours by consumers prevented vulnerable people, who cannot afford to stockpile, to find food (Bakalis et al., 2020; Galanakis, 2020; Hobbs, 2020; Nicola et al., 2020; Pu & Zhong, 2020). Although containment measures have been taken in order to stop profiteering, illicit trade, hoarding of food products (Galanakis, 2020; Petetin, 2020), and food supply was guaranteed, there appears to have been shortages of certain products; in the UK for example, baby formula and flour shelves were rarely replenished (Petetin, 2020). A second barrier to food access was of an economic nature. Panic buying, empty shelves, supply difficulties and sudden spikes in demand have led to an increase in food prices, undermining the food security of the poorest sections of the population (Akter, 2020; Galanakis, 2020; Heck et al., 2020; Save the Children Italia Onlus, 2020). The closure of open-air markets and ban on street-vendors worsened this situation, forcing poor people to shift to more expensive food outlets such as supermarkets or other formal retailers (Devereux et al., 2020). Another side-effect of containment measures was the loss of social measures to fight food insecurity; for instance, school meals, family/grandparent support have stopped (Altieri & Nicholls, 2020; Bakalis et al., 2020; Patrick et al., 2020; Rippin et al., 2020). As result, foodbanks and charities have witnessed an exponential increase in demand of recipients and more and more people have become reliant on their services as a source of food and to prevent hunger (European Food Banks Federation, 2020; FAO, 2020; The Global FoodBanking Network, 2020).

With limited purchasing power, families had to choose less nutritive, cheaper foods. This meant that they predominantly resorted to staple foods, to the detriment of a diet variety and quality (Devereux et al., 2020; Heck et al., 2020; Roberton et al., 2020). This was also caused by the closure of informal markets, which could provide fresh and local foods. The lockdown resulted in an increase of home cooking and artisanal food production, as people bought more staple or simple foods (Bakalis et al., 2020; Pérez-Escamilla et al., 2020). However, it also increased risk of non-communicable diseases and obesity, given the lack of physical activity and the resort to poorly nutritious foods (Abbas et al., 2020; Béné, 2020; Devereux et al.,

2020; Jayawardena & Misra, 2020; Rippin et al., 2020). Stability of food availability and access is affected by COVID-19-related restrictions (Devereux et al., 2020). Persistently unreliable supply chains (inputs to and outputs from farms) would constitute major cause for lasting instability in food production (Savary et al., 2020).

COVID-19 had also affected the sustainability and resilience of food systems. On economic terms, food systems directly employ over 1 billion people (i.e. in food production, processing, food services, distribution delivery, transportation, etc.) (UN SG & UNSDG, 2020). The global crash in demand from hotels and restaurants has seen prices of agricultural commodities drop by 20% (Nicola et al., 2020) and the emerging economic recession are having a profound economic impact putting the jobs and livelihoods of tens of millions at risk (UN SG & UNSDG, 2020). Then, there are global trade restrictions: some countries decided to reduce their food exports to protect national supplies (Devereux et al., 2020). Food production relied on direct trade in food and agricultural inputs, and the trade guaranteed income to millions of people (Savary et al., 2020). However, a highly interconnected and centralized global system is extremely fragile to external shocks (Rippin et al., 2020; Savary et al., 2020). A heavy reliance on complex supply chains, imported food, and just-in-time delivery all contributed to food insecurities during COVID-19 (Pérez-Escamilla et al., 2020). This is evidenced by the food systems inability to cope with sudden and unexpected spikes in food demand, which created short-run stockouts (Hobbs, 2020). On social terms, food systems have failed to provide food security for the most vulnerable sections of the population (i.e. children, women, pregnant woman, elderly, migrants) (Altieri & Nicholls, 2020; Pérez-Escamilla et al., 2020). Migrants, seasonal and informal workers and their families, who already did not have strong safety nets, were even more vulnerable due to job loss or inability to work (Savary et al., 2020; UN SG & UNSDG, 2020). Due to school closure, labour market shocks and rise of COVID-19-related poverty, many children could be forced to work, as household could use child labour to cope with job loss and health issues (ILO & UNICEF, 2020; ILO, 2020). The World Food Programme (WFP) has warned that the number of people facing food crises could double because of the pandemic related issues (Global Network Against Food Crises, 2020). Even if food supply is abundant, people could still face difficulty in access to food (Global Network Against Food Crises, 2020). Some 265 million people in low and middle-income countries will be in acute food insecurity by the end of 2020 unless swift action is taken (World Food Programme, 2020). The World Bank forecasts that by the end of the year 40–60 million more people will be living in extreme poverty (Fleetwood, 2020).

The question that arises is why has COVID-19 had such a deep impact on food security and food systems. Based on data gathered in this paper, we can say that food systems were not sustainable at the outset. The outbreak only exacerbated pre-existing problems (Fleetwood, 2020). First, a large scale, highly centralised, just-in-time supply model, whereby an increasingly small number of farmers supply huge quantities of cheap food that are stored and distributed in supermarkets so as never to interrupt abundance, is prone to falter in the face of shocks (United Nations Environment Programme, 2020). Second, food systems often rely on vulnerable workers (e.g. migrant seasonal workforce) who during the pandemic have been used by national government to cope with shortage of agricultural labour (Neef, 2020; Petetin, 2020). Still, these workers have weak social protection nets and are prone to exploitation, despite their importance in the food production (Fleetwood, 2020; Neef, 2020; Savary et al., 2020). Third, there is evidence that the likelihood of foodborne zoonotic pathogens (FZP) has increased substantially over the past century due to human activities (Galanakis, 2020; Pu & Zhong, 2020). Climate change, soil erosion, intensive use of pesticides, destruction of biodiversity are all issues caused by human activity that increase the risk of FZP outbreaks (Jones et al., 2013). Food systems as conceived are contributing decisively to the overcoming

of planetary boundaries in the fields of biodiversity loss (whose effects have been seen with the COVID-19 pandemic), climate crisis, the nitrogen cycle, deforestation and soil consumption as well as chemical pollution. The more unsustainable food systems are, the more fragile are to external shocks, and the more severe are their consequences for food security. On the other hand, a sustainable food system supports food security, as it makes optimal use of natural and human resources, provides more nutritional food to consumers and is economically fair and environmentally sound (Capone et al., 2014).

Conclusions

COVID-19 has had a negative impact on food security and the sustainability of food systems. The side effects of the pandemic, although little publicised, are central to the stability of the society. The implications are significant: if a virus outbreak affected food security even in industrialized countries, other, more severe shocks could cause food security to collapse. The virus has undermined food security through a combination of decreased food production (due to workforce scarcity, production inputs scarcity and food loss), barriers in access to food (due to physical restrictions, food supply disruption, price increase), and loss of diet nutritional quality and variety. These consequences have affected especially the most vulnerable people, and the health crisis has soon become a social crisis. The pandemic is still ongoing, so food security is still unstable, and the gravity of the repercussions will depend on the governments' success in containing the disease and the course of the pandemic. COVID-19 has also affected food systems, making already unsustainable systems even more so. The ones who lose out are still the most vulnerable sections of societies. COVID-19 has worsened social inequalities, and social crises may rise if food security is not addressed at its roots. From an environmental perspective, COVID-19 has reminded us that there is a close link between food systems, humans and ecosystems. This should lead us to rethink the way we handle nature. Unsustainable food systems are one of the factors leading to increased risk of emerging zoonoses and other exogenous shocks. Making the system sustainable is the only way to avoid future shocks and ensure food security to all sections of the population. The long-standing problems in the food system could be addressed by changing our perspective on how we produce, distribute and consume food, focusing on a more holistic, equitable and environmental-friendly approach. At policy level, it means more integrated policies which should include actions to foster transition towards a sustainable and resilient food system, through a number of practical solutions: localising production and distribution to overcome dependence on long export chains and support local economies; replacing monocultures to increase biodiversity, overcome the pesticide treadmill and enhance soil fertility; seriously implementing the recommendations on climate change mitigation and adaptation. A system that is not overly dependent on exports can withstand the impact of external shocks. Increased biodiversity also means more nutritious and varied foods. Acting against climate change will keep the risk of zoonoses under control and, at the same time, can bring about positive effects on the overall sustainability of food systems. There is also a need for comparative studies, because COVID-19 effects are not the same for every country, although there are some common consequences. These studies should focus on multiple countries and should cover the way to act on food systems sustainability and resilience, and how they can provide food security for all in times of crisis.

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COMMUNITY-SUPPORTED AGRICULTURE IN TIMES OF PANDEMICS

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Abstract

The 2019-2020 coronavirus pandemic is not just about the spread of a disease and the efforts to quarantine it: it is also about the largest global recession in modern history given that about 33% of the global population is placed on lockdown. The pandemic has economic (betting, entertainment, events, financial markets, gambling, HoReCa, manufacturing, publishing, retail, science, technology, tourism, and transportation), educational, political (civil rights, democracy, and sovereignty), religious, and social impacts. Agriculture all over the world is experiencing the negative effects of the pandemic: dairy industry, fishing industry, meat industry, and wine industry are just a few examples. Among economic impact, the retail sector has seen product demand exceeding supply, which resulted in empty retail shelves. Some retailers appealed to contactless home delivery, and an increasing number of small-scale farmers sell their produce directly using digital technologies, turning community-supported agriculture and direct-sale delivery systems into rising ones. This system can make both producers and consumers benefit providing that they observe some (if not all) of ten founding principles of the Teikei System: acceptance of produce, deepening of friendship, democratic management, intended production, learning among each group, maintenance of the appropriate group scale, mutual assistance, mutual concession in the price setting decision, self-distribution, and steady development. This article focuses on the best Teikei System principles to apply during and after the 2019-2020 coronavirus pandemics for the small farmers to survive.

Keywords: *community-supported agriculture, agricultural retail, Teikei System, 2019-2020 coronavirus pandemics impact on agriculture*

Introduction

The 2019-2020 coronavirus pandemic has caused the largest global recession in modern history given that about 33% of the global population has been placed on lockdown. It is clear now that this pandemic has economic (betting, entertainment, events, financial markets, gambling, HoReCa, manufacturing, publishing, retail, science, technology, tourism, and transportation), educational, political (civil rights, democracy, and sovereignty), religious, and social impacts.

Agriculture all over the world has also been experiencing the negative effects of the pandemic: dairy industry, fishing industry, meat industry, and wine industry are just a few examples extensively debated in the media. Among economic impacts, the retail sector has seen product demand exceeding supply, which resulted in empty retail shelves. Some retailers appeal to contactless home delivery, and an increasing number of small-scale farmers sell their produce directly using digital technologies, turning community-supported agriculture (CSA) and direct-sale delivery systems into rising ones. This system can make both producers and consumers benefit provided they observe the ten founding principles of the Teikei System. Successful CSAs operate in fields such as organic agriculture (animal – chickens,

coppice products, livestock, rabbits; meat processing; vegetal – fruits, vegetables) in England (Pilley, 2001) and social farming (individuals with special needs) in England (Pilley, 2001).

Material and Method

The material used in this study consists in literature related to community-supported agriculture (CSA) and to the Teikei System principles from the last two decades. The method used in this study is analytical research, a specific type of research that involves critical thinking skills to identify an assumption and decide if it is true or false (aiming at finding out critical details allowing the addition of new ideas to the study) and the evaluation of the most relevant information relative to the research being carried out. Thus, data and other important facts related to the study have been compiled and, after the information has been collected and evaluated, the sources investigated have been used to support the main idea of the study – the need to apply the Teikei System principles during and after the 2019-2020 coronavirus pandemic for the small-farmers to survive.
Numerous sources provide counselling and examples of good practices (case studies) in the field of community supported agriculture (Wilkinson, 2001; Gregson & Gregson, 2004; Wright, 2005; Martin et al., 2015; Adam, 2016; Volz et al., 2016; Ernst, 2017; McMurray, Hall & Brain, 2017; Sylla, et al., 2017; Telles, et al., 2017; Volz et al., 2017; Woods, et al., 2017).

Results and Discussion

1. Community Supported Agriculture
The concept of community supported agriculture (CSA) – encompassing a broad range of partnerships (partnership designates a relationship based on mutual trust, openness, shared risk and shared rewards) between consumers and producers – is not a new one in agriculture: it was developed in the 1970s in Japan by a group of women concerned about the increase of food imports and the loss of local farming, who established a growing and purchasing relationship (Japanese teikei, "putting the farmer's face on food") with local farmers (Wright, 2005). This concept reached Europe and then the USA, where it was named community supported agriculture or CSA. A variety of similar production and marketing systems are in use worldwide: Brazil – Organizações Rurais de Economia Solidária; Bulgaria – Съпричастно земеделие; Canada – Agriculture soutenue par la communauté; Croatia – Grupa solidarne razmjene; France – Association pour le maintien d'une agriculture paysanne; Germany – Solidarische Landwirtschaft; Italy – Gruppi di Acquisto Solidale; Japan – Teikei; Norway – Andelslandbruk; Portugal – Reciproco; Romania – Asociația pentru Susținerea Agriculturii Țărănești (Telles, Macedo & Messias Bittencourt, 2017).
It is, nowadays a partnership between a farmer and a community of supporters aiming to establish agriculture on a completely new system in which a sense for community could be experienced even in matters of economics, human and natural resources are not exploited, production is based on acknowledged needs, reason prevails instead of the chance of the marketplace, and self-interest is balanced with an interest in fellow human beings.
Th difference between a CSA farm and a traditional farm was made clear by Polimeni et al. (2006, 21): "the amount of money and time spent on marketing and retailing. A traditional farm spends countless hours and money on marketing and advertising. Furthermore, a CSA does not have this problem because the majority of members learned about the CSA through word-of-mouth, not formal advertising."
Bîrhală and Möllers (2014a, 2014b) and Moellers and Bîrhală, 2014) analysed the costs and benefits for both sides, the farmers and the consumers from Western Romania, when entering

into a direct, trust-based market relationship in the form of CSA, from the perspective of solidarity economy.

The last twenty years have seen, at least until a few years ago, a revival of the idea of a CSA – both theoretically and practically.

According to the organisers, there are several types of CSAs depending on leadership and ownership (Adam, 2016, 2): farmer-driven/managed or producer/subscription-led/run, consumer/shareholder/subscriber-driven, community-led/owned or farmer co-operative, farmer-consumer/shareholder/subscriber cooperative or producer-community partnership, consisting in a consumer-farmer-driven CSA with two or more farms (in which land, other resources and work may be co-owned by farmers and consumers) co-operating to produce and distribute food (UK).

CSA members are satisfied with the following CSA traits: freshness, season length, connection to community/farm, variety, sense of community, ease of delivery/pickup, price, and size of share. CSA appealed to farmers because of the turn to more sustainable farming methods, of the creation of "local" and "shorter" food supply chains; and of new forms of discerning and reflexive consumerism. Perez, et al., (2003) claimed that most important reasons why farmers want to become CSA members are organic produce, support or buy local, fresh produce, support organic (farms/farmers/agriculture), quality produce, convenience, support small or family farms/farmers, health, variety, good price/value, support sustainable agriculture, eat seasonally, know how/where food was grown, etc. Brehm and Eisenhauer (2008) found that the motivations for joining CSAs are their strong desire for affordable food, for food free of pesticides, for food that tastes better than what I can find in a local grocery store, for locally-grown food products, for food that is not genetically engineered, for fresh food, for organic food products, and for food that is easily accessible; their strong desire to develop a stronger sense of community, to eat food products that are in season, to reduce packaging on food products, to support local community members who grow food, to support sustainable agriculture, to support community's local economy, to know where and how food is grown, and to meet new people who care about where their food comes from; and specific health reasons/conditions that require this kind of food.

The limitations of CSA are fourfold – agricultural climate, consumer trends, policy, and skills (Pilley, 2001). Lass et al. (2001) found that American CSAs faced challenging financial situations, in general, and that farmers were unsatisfied than were satisfied with their ability to cover farm costs, their community involvement, their compensation and financial security, their level of stress and quality of life, and workloads for them and their workers. According to Perez, et al., (2003) the reasons why CSA members might/will not renew their membership are product mix issues (more variety, want to select own), problems with quantity (threw out too much, etc.), household issues (moving, don't cook, etc.), cost/value issues, pick-up issues, problems with quality, problems processing and storing, preferring farmers markets, etc. Some members leave CSA farms because they are out of town or moved away, they have their own garden, they have no time, there is too much food, they got the wrong vegetables in limited seasons, farms changed, hanging farms, the poor quality of the produce and produce were not affordable.

2. Community-Supported Agriculture and the Covid-19 Pandemic

According to FAO (2020), the impact of the COVID-19 pandemic on food and agriculture is huge: the truth is that mankind risks a looming food crisis – border closure, market disruptions, quarantines, supply chain disruptions (challenges in terms of logistic bottlenecks caused by the basic aversion behaviour of the consumers, traders and workers and by the restrictions of movement), and trade disruptions have restricted people's access to diverse, nutritious and sufficient sources of food, particularly in countries already affected or hit hard

by the virus. Agricultural production has been affected by closure of restaurants, cafĕs and street food outlets, less frequent grocery shopping (which diminished the demand for fresh produce and aquaculture/fisheries products), restrictions on tourism, school meals suspension, shortage of fertilisers, veterinary medicines, etc.

Food security and livelihoods most at risk have been in the 53 countries home to 113 million people experiencing acute severe food insecurity, in the 44 countries in need for external food assistance, in the countries relying heavily on primary exports like oil, as well as in the vulnerable communities already suffering from other crises – including fishers, pastoralists, and small-scale farmers who have been hindered from accessing markets to buy essential inputs or sell their products, caring for their livestock, fishing, working their land, and who have been fighting higher food prices, income losses, job losses, limited purchase power.

The impact of the COVID-19 pandemic on food production, agricultural and aquaculture/fishery supply chains (agricultural inputs, marketing, processing, storage, and transportation; consumers; fishery inputs, marketing, processing, storage, and transportation) and markets have not been critical so far: food supply has been adequate, global cereal stocks are at comfortable levels, markets have been stable, and the outlook for wheat and other major staple crops is positive. Blockages to transport routes and quarantine measures have been particularly obstructive for fresh food (e.g. eggs, fruits and vegetables) urban supply and demand chains, causing food loss and waste, impeding farmers' access to markets and, thus, curbing their productive capacities and hindering them from selling their produce.

Fear of contagion causes reduced visits to food markets – hence, increased e-commerce deliveries, lower restaurant traffic, and a rise in eating at home – and control of cargo vessels puts fresh fruits and vegetables at risk.

Some food commodity prices have increased at local markets because of local logistic problems but, as the downward demand shock increased, prices went down affecting rural farmers.

To avoid disruptions in the food supply chain and food production, FAO (2020) recommended all countries to:

- Keep international trade open and take measures that protect their food supply chain (from obtaining inputs such as seeds to assuring smallholder farmers have access to markets to sell their produce);
- Focus on the needs of the most vulnerable, and scale up social protection programmes including cash transfers;
- Keep their domestic food supply value chains alive and functioning;
- Taking all necessary precautions, seeds and planting materials must continue to flow to smallholders; animal feed to livestock breeders; and aquaculture inputs to fish farmers. Agricultural supply chains should be kept alive by any means compatible with health safety concerns;
- Maintain agricultural activities.

Yet, another solution to support agriculture, in general, and food supply chains, in particular, in times of crisis, is to try to apply the ten principles of the Teikei System (JOAA, 1993) that best meet the needs caused by the COVID-19 pandemic. Given the circumstances created by this pandemic, the following principles should be applied by both producers and consumers to fight its negative effects on agriculture:

1. The principle of mutual assistance means that both groups should help each other by providing labour and capital to support their own delivery system with delivery stations (drop off points), where the nearest consumers of 3 to 10 families can get the delivered products – to fight the fear of contagion, the lack of access to markets, the quarantines, and the restrictions of movements;

2. The principle of intended production means that producers should, through consultation with consumers, intend to produce the maximum amount and maximum variety of produce within the capacity of the farms – to fight the lack of food of high-value commodities (dairy produce, eggs, fruits and vegetables);
3. The principle of accepting the produce means that consumers should accept all the produce that has been grown according to previous consultation between both groups, and their diet should depend as much as possible on this produce – to fight food loss and waste;
4. The principle of mutual concession in the price decision means that producers should take full account of savings in labour and cost, due to the curtail of grading and packaging processes and to the acceptance of all their produce, while consumers should take into full account the benefit of getting fresh, safe, and tasty foods – to fight, first, higher food prices (caused by the supply chain disruptions) and, later, low food prices (caused by the decrease of the purchase power);
5. The principle of deepening friendly relationships between the two groups through maximizing contact between the partners provided social distancing rules (cloth face covering, a distance of 2 m between people) are observed;
6. The principle of self-distribution means that the transportation of produce should be carried out by either group, up to the drop off point, without dependence on professional transporters – to fight blockages to transport routes and supply chain disruptions;
7. The principle of democratic management means that both groups should avoid over-reliance upon limited number of leaders in their activities, and try to practice democratic management with responsibility shared by all – to fight all the inconveniences caused by the COVID-19 pandemic;
8. The principle of learning among each group means that both groups should attach much importance to studying among themselves and try to keep their activities from ending only in the distribution of safe foods – to fight all the inconveniences caused by the COVID-19 pandemic;
9. The principle of maintaining the appropriate group scale means that increasing membership should be promoted through increasing the number of groups and the collaboration among them – to fight all the inconveniences caused by the COVID-19 pandemic;
10. The principle of steady development means that both groups should choose promising partners, even if their present situation is unsatisfactory, and to go ahead with the effort to advance in mutual cooperation – to fight all the inconveniences caused by the COVID-19 pandemic.

Conclusions

After analysing the concept of community-supported agriculture, the principles of the Teikei System, and the impact of Covid-19 pandemic on food and agriculture, it is clear that all ten principles of the Teikei System can help small farmers fighting the negative impacts of the Covid-19 pandemic. Thus:

Principles 1-4 and 6 of the Teikei System can help fighting particular inconveniences caused by the COVID-19 pandemic (blockages to transport routes, fear of contagion, food loss and waste, high food prices, lack of access to markets, lack of food of high-value commodities (dairy produce, eggs, fruits and vegetables), low food prices, quarantines, restrictions of movements, and supply chain disruptions);

Principles 7-10 of the Teikei System can help fight all the inconveniences caused by the COVID-19 pandemic;
Principle 5 of the Teikei System can be applied provided social distancing rules are observed.

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AGRI-ENVIRONMENTAL INDICATORS AS A TOOL FOR CLASSIFYING EUROPEAN UNION MEMBER COUNTRIES

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Abstract

Agriculture plays a significant role in the field of sustainable development. For this reason, it is clear why recent research in the field of sustainability is related to the topic of sustainable agriculture. There are many definitions of sustainable agriculture in the literature, but they are all based on three basic principles: ecological stability, economic sustainability and social acceptability. In general, global sustainability and even global sustainability in agriculture, cannot be achieved until all the actors become aware of their significant role in such a vital project. Therefore, it is necessary to constantly monitor the achievements of the goals of sustainable agriculture. Many institutions offer different sets of indicators to observe the achievement of the given goals of sustainable agriculture. Most of them offer a set of indicators to analyze only the ecological aspect of sustainable agriculture. In this study, the authors opted for a set of agri-environmental indicators provided by Eurostat which offers 12 headline indicators and 62 sub-indicators that further explain headline indicators. Given that the data for individual indicators are incomplete, this study included 8 headline indicators and the corresponding 12 sub-indicators. Based on a set of 12 selected indicators, a cluster analysis was conducted for the European Union (EU) member countries for 2016. The results of analysis indicated that observed countries were grouped into 6 homogeneous clusters. After grouping countries, additional analysis of indicators by cluster was undertaken, attempting to identify agri-environmental areas where countries of the same group are lagging behind.

Keywords: *agri-environmental indicators, cluster analysis, European Union, sustainable agriculture.*

Introduction

The concept of sustainable agriculture has recently gained in importance, which is confirmed by numerous studies (Janker et al., 2018; Sing, 2015; Moore, 2015; Pant, 2014; Pretty, 2008; etc.) which are concentrated around this topic in a direct or indirect way.The main reason for this trend is that agriculture is one of the most important pillars of society development and is the key to long-term socio-economic development of any country(SASB, 2019). Therefore, the purpose of research on sustainable agriculture is to raise the awareness of individuals and all those directly involved in the system of cultivation, processing, distribution and consumption of food that they can play a significant role in ensuring a sustainable agricultural system.The term sustainable agriculture means an integrated system of plant and livestock production practices that have high economic value, meet long-term food needs, improve the quality of life of farmers, local communities and society as a whole, while preserving the quality of the environment and natural resources (FAO, 2019). Consequently, the general goal of sustainable agriculture is to meet the food and textile needs of society at present, without compromising the ability of future generations to meet their own needs (UCDavis - Agricultural Sustainability Institute, 2019). Achieving the overall goal implies meeting the sustainability goals through three aspects: environmental aspect, economic aspect and social aspect. None of these aspects should be neglected, because otherwise the realization of the

remaining two, and thus the realization of the sustainability of agriculture in general, is jeopardized.A group of authors managed to prove that by measuring sustainabilityof Polish farms based on FADN data (Sulewski et al., 2018). In order to measure sustainability in general and therefore sustainability in agriculture, as well,there have to be indicators that enable monitoring of its individual segments. In this regard, a number of organizations propose sets of indicators to measure the achievement of sustainable agriculture goals. Latruffe et al. (2016) point out that the ecological aspect of sustainable agriculture has experienced an "indicator explosion". The reason for this is that, lately,society as a whole, is interested in the ecological dimension of sustainable development, including sustainable agriculture. According to Brown et al. (1987) since 1980, environmental issues have become the basis of sustainable agriculture and short-term goals of this dimension grow into long-term goals, so for example the protection of natural resources and ecosystems is a goal that is relevant to this day (Pretty, 2008). In thispaper, only the environmental aspect of sustainable agriculture is observed due to existence of already determined set of indicators from several sources. The authors chose to base their research on a set of 12 headline agri-environmental indicators of European Union countries offered by Eurostat. In addition to the headline indicators, 62 sub-indicators appear in the Eurostat set, which further explain the headline indicators. Their organization is presented in Table 1.

Table 1. Agri-environmental indicators by Eurostat (Eurostat, 2019)

Ordinal number	Headline indicators	Unit of measure	Number of sub-indicators
1.	Area under organic farming	% of utilised agricultural area	0
2.	Sales of fertilisers by type of nutrient	Tonnes	3
3.	Sales of pesticides by type of pesticide	kg of active ingredient	35
4.	Share of irrigable and irrigated areas in utilised agricultural area	% of utilised agricultural area	2
5.	Final energy consumption by agriculture/forestry per hectare of utilised agricultural area	kgOE/ha	0
6.	Share of main land types in utilised agricultural area	% of utilised agricultural area	3
7.	Share of main livestock types in total livestock units	% of LSU	6
8.	Livestock density index	Livestock units per ha	0
9.	Gross nutrient balance on agricultural land	kg/ha	2
10.	Ammonia emissions from agriculture	Tonnes or kg/ha	0
11.	Ammonia emissions from agriculture - % of total emissions	% of total emissions	0
12.	Greenhouse gas emissions from agriculture	% of total emissions	11

Materials and methods

Based on Table 1, it can be seen that the agri-environmental indicators set has 74 indicators. However, for the purposes of our analysis, a set of 12 indicators has been prepared, with the help of which the European Union countries will be grouped into homogeneous groups, having in mind the ecological aspect of sustainable agriculture.The year 2016 was analyzed because it is the last year for which there were data for the largest number of indicators for all countriesobserved. Consequently, a significantly smaller number of indicators were included in the survey compared to the set offered in the Eurostat database.For example, the headline indicator "Sales of fertilizers by type of nutrient" was not taken into analysis because there were no data for three European Union countries: Belgium, Luxembourg and Malta.Furthermore, data for the headline indicator "Share of irrigable and irrigated areas in

utilized agricultural area" were not available for Luxembourg, so this indicator was also excluded from the analysis. In addition to these two indicators, the headline indicators "Livestock density index" and "Gross nutrient balance on agricultural land" were excluded from the analysis because the last available data for all EU countries were in 2013 and 2015, respectively. Table 2 presents the indicators with which the cluster analysis was conducted.

Table 2. Agri-environmental indicatorsincluded into the analysis (Authors' own work)

Ordinal number	Indicators selected	Headline indicator
1.	Utilised agricultural area excluding kitchen gardens	Area under organic farming
2.	Herbicides. haulm destructors and moss killers	Sales of pesticides by type of pesticide
3.	Final energy consumption by agriculture/forestry per hectare of utilised agricultural area	Final energy consumption by agriculture/forestry per hectare of utilised agricultural area
4.	Arable land	Share of main land types in utilised agricultural area
5.	Permanent grassland	
6.	Permanent crops	
7.	Live bovine animals	Share of main livestock types in total livestock units
8.	Live sheep	
9.	Live poultry	
10.	Ammonia emissions from agriculture	Ammonia emissions from agriculture
11.	Ammonia emissions from agriculture - % of total emissions	Ammonia emissions from agriculture - % of total emissions
12.	Greenhouse gases (CO2, N2O in CO2 equivalent, CH4 in CO2 equivalent, HFC in CO2 equivalent, PFC in CO2 equivalent, SF6 in CO2 equivalent, NF3 in CO2 equivalent)	Greenhouse gas emissions from agriculture

Cluster analysis is a method of multivariate analysis used to group objects according to multidimensional criteria into groups, while trying to maximize internal homogeneity and external heterogeneity.

The starting point for different classification methods is the data on objects and their characteristics arranged in the data matrix X dimension*nxp*, consisting of*n*rows (which represent the number of observed objects) and *p*column (which represent the number of variables). Based on the data matrix X, we form a matrix of proximity, dimension *nxn*, the elements of which measure the degree of similarity or difference between all pairs of observation units (Kovačić, 1994). There are a large number of measures that quantify the distance between the observed objects according to all analyzed features. In this paper, we use the square Euclidean distance obtained according to the following formula (Kovačić, 1994):

$$d_{rs}^2 = \sum_{j=1}^{p} \left(x_{rj} - x_{sj}\right)^2, \qquad (1)$$

where x_{rj} and x_{sj} are values of indicator j for observed objects r and s,respectively.

On the basis of proximity measures, numerous procedures for grouping elements have been developed, which can be classified into two groups: hierarchical and non-hierarchical methods. Hierarchical methods are more often used in the literature, and most often Ward's method of connection. Ward's connection method is based on the intergroup sum of squares. Using this method, two groups merge into one if their association resulted in the smallest increase in the sum of squares between groups in relation to the increase that would occur in the case of merging any two groups (Kovačić,1994).

Results and Discussion

In this paper, the states members of the European Union are grouped into homogeneous groups according to agri-environmental indicators for 2016, which are shown in Table 2. In order to eliminate the effect of different units of measurement, the data are standardized, using the following relations:

$$Z_{rj} = \frac{X_{rj} - \overline{X}_j}{S_j}, \qquad (2)$$

Random variable Z_{rj} has a standardized normal distribution with arithmetic mean 0 and variance equal to 1.

A cluster analysis model based on the Euclidean square distance between the analyzed countries and hierarchical grouping based on Ward's connection method was chosen. To determine the level of intersection of the hierarchical tree, the simplest procedure was used, which is based on monitoring the distance at which two classes merge into one. Namely, if a significant change in the value of the distance between classes is observed in an iteration, then the number of clusters that preceded this iteration is considered optimal(Figure 1).

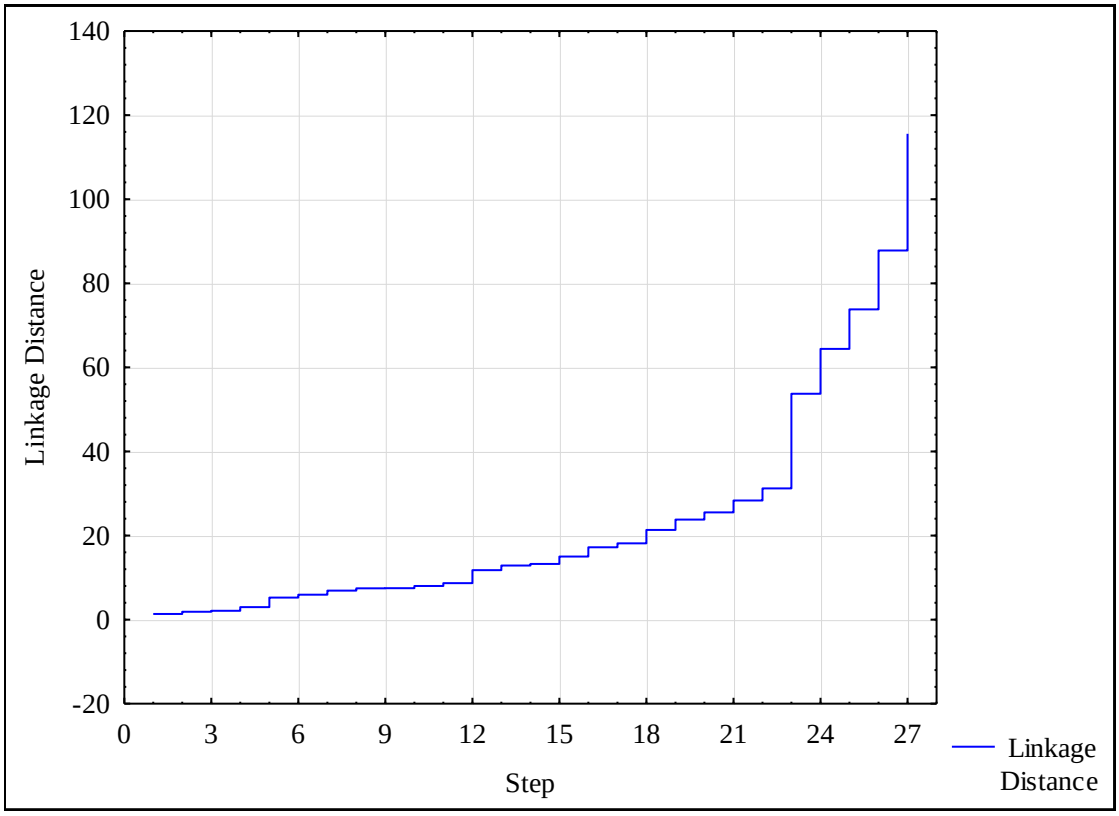


Figure 1. Values of the distance measure between the groups (Authors' own work)

Based on Euclid's function, as a synthetic indicator of the closeness of the observed countries to the indicators of sustainable agriculture, the most similar countries are Slovakia and the Czech Republic, followed by Lithuania and Latvia, Croatia and Bulgaria, then Sweden and Estonia. Furthermore, according to the similarity in the level of achieved goals of sustainable agriculture, Romania joins Croatia and Bulgaria. The following steps and levels of hierarchical grouping based on Ward's connection method indicate that the observed countries can be grouped into six clusters based on the achieved goals of sustainable agriculture (Figure

2). Therefore, it can be concluded that the countries of the European Union are not homogeneous on this issue.

The distribution of European Union countries according to individual clusters is presented in Table 3. The first cluster includes countries that have access to the sea and they are: Italy, Spain, France, Poland and Germany. The second and fifth clusters include only one country, the Netherlands and Ireland, respectively. The third cluster consists of three countries: Hungary, Malta and Cyprus.The fourth cluster includes: Greece, Portugal, Great Britain, Romania, Croatia and Bulgaria, while the sixth cluster is the largest in terms of the number of countries it covers and consists of the following 12 countries: Slovenia, Luxembourg, Lithuania, Latvia, Denmark, Finland, Sweden, Estonia, Austria, Slovakia, the Czech Republic and Belgium.

According to the data in Table 4, which shows the average values for each observed indicator of sustainable agriculture according to the obtained clusters, interesting conclusions can be drawn. The fifth cluster with only one country - Ireland - stands out as the cluster with the worst average values of the indicatorsobserved. Ireland has the worst position when it comes to indicators related to climate change. It is also the country with the lowest percentage of area under organic production as well as the lowest percentage of arable land and the percentage of used agricultural land under permanent crops. On the other hand, the third cluster, which includes two small countries, Malta and Cyprus, stood out as a cluster with several best average values of the observed indicators. Among them, perhaps the most important is to single out the indicators related to the emission of harmful gases from agriculture. In the third cluster, the average percentage of arable land is the highest as well as the average percentage of used agricultural land under permanent crops, and also the highest percentage of live poultry. Acording to the OECD (2013) document, the countries that had the largest share of land under organic production in the period from 2008 to 2010 are Austria, Sweden, Czech Republic, Estonia, Slovak Republic and Finland. This means that the situation in 2016 has not changed significantly compared to the period of six years ago, because the mentioned countries, according to our research, belong to the sixth cluster, which according to Table 4, is the best, having in mind the indicator *area under organic farming* described by the indicator *utilized agricultural area excluding kitchen gardens*. Accordingly, the countries of the fourth cluster have the best position in terms of the amount of pesticides sold.

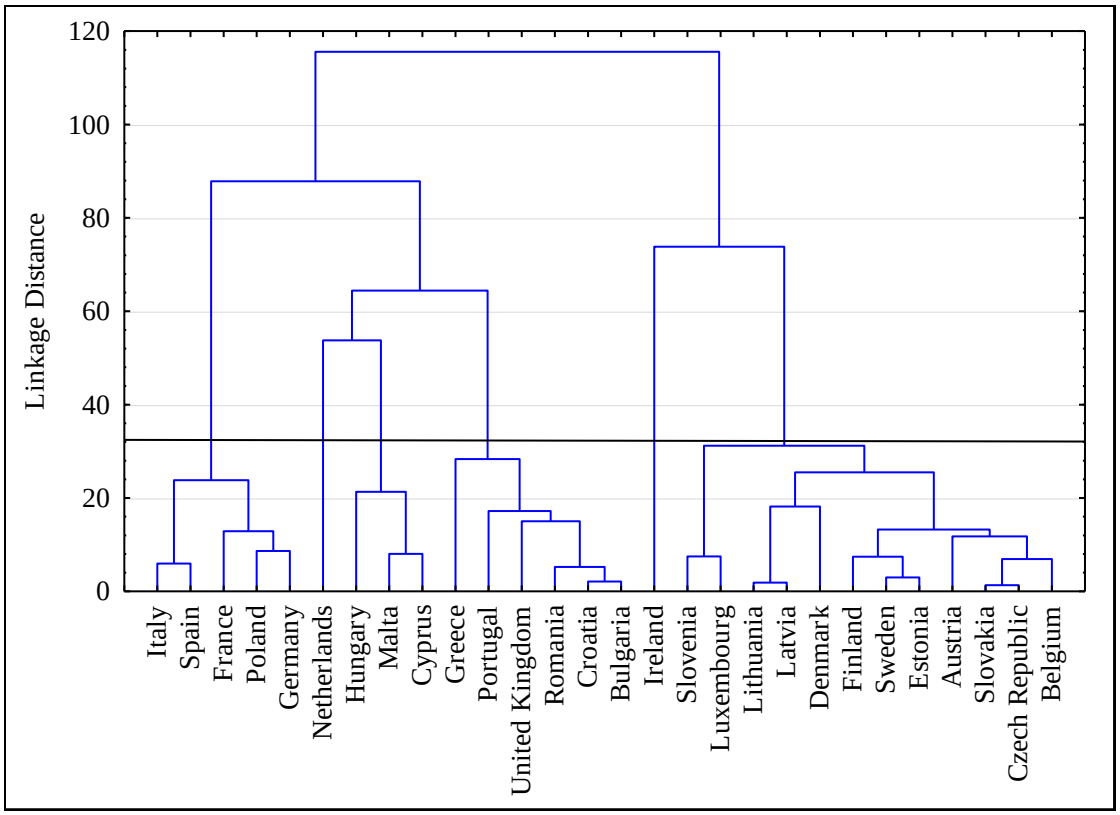


Figure 2. Dendrogram of 28 EU Countries for the Year 2016. (Authors' own work)

Table 3. Distribution of EU countries by clusters(Authors' own work)

I cluster	II cluster	III cluster	IV cluster	V cluster	VI cluster
1. Italy 2. Spain 3. France 4. Poland 5. Germany	1. Netherland	1. Hungary 2. Malta 3. Cyprus	1. Greece 2. Portugal 3. United Kingdom 4. Romania 5. Croatia 6. Bulgaria	1. Ireland	1. Slovenia 2. Luxembourg 3. Lithuania 4. Latvia 5. Denmark 6. Finland 7. Sweden 8. Estonia 9. Austria 10. Slovakia 11. Czech Republic 12. Belgium

Table 4. Average values of selected indicators by clusters (Authors' own work)

Selected indicators	Clusters					
	I	II	III	IV	V	VI
Utilised agricultural area excluding kitchen gardens	7,66	2,91	2,88	4,50	1,72	11,58
Herbicides. haulm destructors and moss killers	16.097.043,20	2.745.753,00	1.581.191,00	3.545.962,50	2.243.469,00	1.304.964,67
Final energy consumption by agriculture/forestry per hectare of utilised agricultural area	136,91	2.030,71	314,63	69,08	46,61	193,15
Arable land (% of total utilised agricultural area)	63,46	57,20	79,53	49,18	9,40	68,50
Permanent grassland (% of total utilised agricultural area)	27,90	40,60	5,33	42,30	90,60	30,15
Permanent crops (% of total utilised agricultural area)	8,54	2,10	12,80	8,18	0,00	1,32
Live bovine animals (% of total livestock units)	47,10	44,20	27,60	40,58	82,40	59,34
Live sheep (% of total livestock units)	4,56	1,10	8,13	19,20	8,30	2,13
Live poultry (% of total livestock units)	18,32	17,40	25,10	15,85	1,80	11,60
Ammonia emissions from agriculture (tonnes)	462.748,80	109.720,00	28.570,33	93.904,67	114.931,00	36.277,67
Ammonia emissions from agriculture - % of total emissions	93,54	85,70	94,10	86,00	99,00	90,63
Greenhouse gas emissions from agriculture	9,76	9,10	6,33	10,85	29,80	11,78

Conclusions

According to the acquired results we can conclude that the countries of EU are heterogeneous when it comes to answering the question of ecological aspects of sustainable agricultura. This is also supported by the fact that there are two clusters containing just one country. One of those countries is Ireland, that cluster is ranked the lowest. Another interesting result shows that every cluster of countries stands out by some indicators as a cluster with the best average values.Authors of research on the possibilities of measuring sustainable agriculture and reviewing indicators for these purposesLatruffe et al. (2016) point out that when choosing indicators, researchers must be careful because the selected indicators will influence the analysis and final conclusions. In that sense, one should be aware that by choosing another set of indicators, we could get a different way of grouping the countries of the European Union and report different conclusions. Finally, it should be borne in mind that indicators for measuring sustainability in general, and in agriculture, as well, are not perfect (Bell and Morse, 2018) because they have a difficult task, to measure something that has a dynamic character(Ikerd, 1993).

Acknowledgement

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FACTORS DETERMINING ACCESS TO CREDIT BY ORANGE FARMS OWNERS IN TUYENQUANG PROVINCE, VIETNAM

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Abstract

While Vietnam started as a poor agricultural country, her economy has improved in recent years. Economic development has changed the country significantly in all aspects, especially agriculture, rural areas and peasant living standards. Orange and farming models have been encouraged in many localities in Vietnam for a higher income and living standards of the farmers who account for a large proportion of Vietnamese labor structure. Access to credit is one of the essential keys to decrease poverty, promote production, and smoothly increase consumption. However, access to credit by the farm owners has currently got constraints. This paper was identified factors determining access to credit by orange farm's owners in TuyenQuang Province, Vietnam. The study randomly chose 107 farm owners in the study site to interview. The primary data were collected from April to June 2019 by using a structured questionnaire. The Probit model regression was applied to identify the factors. The independent variables were age of farm owners, education level, family size, family labor, farming experience, farm size, social capital, and farm income. The results indicated that the age of farm owners, education level, family size, orange cultivation experience, farm size, and social capital had significant influence on the access to credit of farm owners. Cooperative and intensive extension systems are two recommendations for local authorities and plan makers to improve orange farm owners' access to credit.

Keywords: *Access to credit, Social capital, Orange production, Probit model, Vietnam*.

Introduction

Starting as a poor agricultural country, the Vietnamese economy has flourished in recent years. The proportion of the population in urban areas continuously shows an upward trend, while the population in rural areas experiences an opposite direction (General Statistics Office of Vietnam, 2019b). The majority of Vietnam's population lives in rural areas and do farming as a livelihood. In 2018, the urban population was 33.8 million persons, accounted for 35.7%; the rural population was 60.9 million persons, made up 64.3% (Tuyen Quang Statistic Office, 2019). The agriculture sector created jobs for 67.4% labor force and is still the main economic sector which contributed 14.7% to Vietnamese gross domestic product (General Statistics Office of Vietnam, 2019a). Agriculture, farmers and rural areas are particularly interested in the process of industrialization and modernization by the Vietnamese government (National Assembly of the Socialist Republic of Vietnam, 2016). Restructuring agriculture and rural areas towards industrialization and modernization; augmenting incomes per day of labor and the one on a hectare of farmland; improving living standards for farmers are the principal goals of Vietnam's sustainable development period 2011-2020 (Prime Minister of Socialist republic of Vietnam, 2012). Access to credit is one of the essential keys to decrease poverty, promote production, and smoothly increase consumption (Claessens, 2006). However, farmer

household access to credit in rural areas of Vietnam remains restrictions like poor and low income, lack of collateral security, small land size (Doan, 2015; Khoi, Gan, Nartea, & Cohen, 2013; Vuong Quoc, Marijke, Jacinta, Le Long, & Luc, 2012). Farming model has been encouraged to develop in Vietnam since the 2000s (Government of the Socialist Republic of Vietnam, 2000). At the same time, orange trees have become the spearhead of several localities. TuyenQuang province is one of fourteen provinces in the region of Northern Midlands and Mountainous of Vietnam where both farming-model and orange cultivation have been inspired by development. Orange trees have been cultivated in large volume in TuyenQuang for two decades, positively contributed to national orange production. Orange cultivation has created jobs for rural people, augmented the living standards of the growers, and improved local socio-economic factors (People's Committee of Ham Yen District, 2015, 2016, 2017, 2018, 2019). Nevertheless, access to credit by the farm's owners has still got constraints. Unfortunately, there has been no study reported on access to credit by orange farm households. Hence, this study focused on identifying the determinants of access to credit by orange farm's owners in TuyenQuang Province, Vietnam. The study also aims to provide policy recommendations to the plan makers for taking appropriate actions towards augmenting access to credit by the orange-farm owners.

Material and Methods

Study Site and Sampling

Tuyen Quang is a mountainous province where about 165 km from Hanoi (Tuyen Quang Portal, 2018). Agriculture, forestry, and fisheries contributed to 24.28% of TuyenQuang's gross regional domestic product in the year of 2018 (Tuyen Quang Statistic Office, 2019). The study chose Ham Yen District as the study site because all orange farms of TuyenQuang province placed there. Figure 1 described the study site.

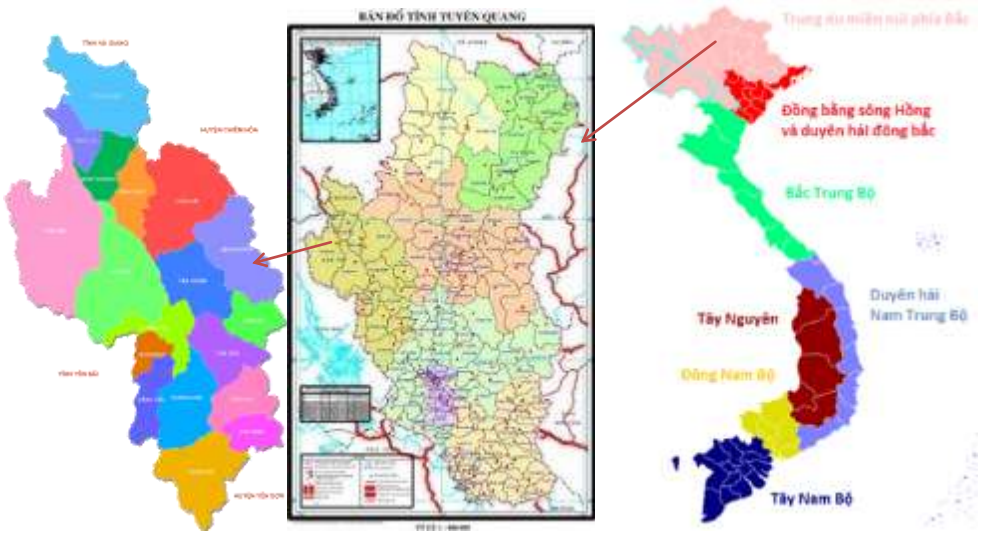


Figure 1. The study site

According to the Vietnamese Law, the farm needs to meet all two criteria: annual production value minimum of VND 700 million and the areas at least 2.1 hectares to obtain the Farm certificate (Ministry of Agriculture and Rural Development of Vietnam, 2011). The experimental unit is a specific farm that meets the criteria of the farm. Due to time and cost constraints, the sample size needs to be determined (Bryman & Bell, 2011). The random sample selection method was chosen (Kothari, 2004). The number of 107 samples were

surveyed. After document reviews and trial interviews, a structure questionnaire was formed(Cooper & Schindler, 2011). Official interviews took place from April to June 2019.

Analytical Methods

The study used descriptive statistics and inferential statistical tools. The descriptive statistics were frequency counts, percentages, and mean; the inferential statistical tool was the Logit regression model. To identify the relationship between independent variables and a dichotomous dependent variable, the Logit model recommended (Cox & Snell, 1989; Greene, 2008). The access to credit by orange farm's owners is a dichotomous dependent variable written as follows:

$$y_i = \begin{cases} 1\ if\ farm\ owner\ access\ to\ credit \\ 0\ if\ farm\ owner\ no\ access\ to\ credit \end{cases} \quad \textbf{(1)}$$

The binary logistic regression explored the socio-economic factors influencing access to credit by orange farm owners. Accordingly, a set of independent variables chosen to determine their impacts on access to credit by orange farm owners in the study areas. The model specified by:

$$Y_i/_{1-Y_i} = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \beta_4 X_{4i} + \beta_5 X_{5i} + \beta_6 X_{6i} + \beta_8 X_{7i} + \beta_8 X_{8i} + \varepsilon_i \quad (2)$$

Where the denote imeans the i-th farm; Y is the probability that an orange farm's owner has access to credit; (1-Y) is the probability that an orange farm's owner does not have access to credit; β0 is the intercept term and β1, β2, …, β8 are the coefficients of the independent variables X1, X2, …, X8; εi denotes the random error term.

Eightsocio-economic independent variables named age, education level, family size, family labor, experience, farm size, social capital, and farm income. The variables were defined as in Table 1:

Table 1: Descriptions, measurement, and expected sign of independent variables

Independent variables		Descriptions	Measurement	Expected sign
AGE	**Age**	**Age of farm owner**	**In years**	**+**
EDU	**Education level**	**Level education of farms' owner**	**In schooling years**	**+**
FSZ	**Family size**	**Number of family members**	**Persons equivalent**	**+/-**
FLB	**Family labor**	**Number of family members working on the farm**	**Persons equivalent**	**-**
EXP	**Experience**	**Experience in orange cultivation of the farms' owner**	**In years**	**+**
AREA	**Farm size**	**Total orchard areas cultivated of the farm**	**In hectares**	**+**
SOCI	**Social capital**	**Social capital of the farm owner**	**1= a member of a group, 0 = otherwise**	**+**
INC	**Farm income**	**The previous year's farm income**	**In million VND**	**+/-**

Results and Discussion

The study showed that 75 observed farms owner had accessed credit while 32 did not. Using the model (2) to predict the farm's owner access to credit. In 75 farm's owners had accessed credit, the model predicted 70 farmers had accessed credit with the percentage correct of 93.3%; and in 32 farms owners had not accessed credit, it predicted 28 farmers had not accessed credit with the percentage correct of 87.5%. The overall percentage correct prediction was 91.6%. The binary regression results of variables influencing access to credit shown in Table 2. The results indicated that the model with all the variables entered in step 1: age, education level, family size, family labor, farming experience, farm size, social capital, and farm income had goodness-of-fit (with sig. = 0.892), and was equally consistent with theory. It is evident from Table 2, the coefficients of age, education level, family size, farming experience, farm areas, and social capital were all found to be significant at the various levels of significance ($p < 0.01$, $p < 0.05$, and $p < 0.1$). The estimated factors which had a positive impact include age, education level, farming experience, farm area, and social capital. Family size was the factor that had a negative impact on farm owners' access to credit.

Table 2: Binary regression results of orange farm's owners' access to credit

Independent variables	B	S.E.	Wald	Sig.
AGE	0.100	0.060	2.744	0.098***
EDU	0.627	0.193	10.530	0.001*
FSZ	-0.995	0.451	4.878	0.027**
FLB	0.506	0.423	1.436	0.231
EXP	0.314	0.104	9.119	0.003*
AREA	0.487	0.212	5.291	0.021**
SOCI	2.907	0.854	11.587	0.001*
INC	0.000	0.000	1.576	0.209
Constant	-16.310	4.348	14.074	0.000

(*, ** and * represent level of significant at 1, 5, and 10 percent respectively)**

The results of age and education level of farm owners corroborated the research findings of Zeller in Madagascar, Kosgey in Kenya, and Doan in Vietnam (Akpan, Patrick, Udoka, Offiong, & Okon, 2013; Doan, 2015; Kosgey, 2013; Zeller, 1994). The result of experience aligned with the researches in Pakistan of Saqib et al., of Ali and Khattak (Ali & Khattak, 2011; Saqib, Kuwornu, Panezia, & Ali, 2018). It has explained that higher age, higher education level implied better technical knowledge, know-how, and farming experience getting more chance to access to credit. The result of farm size was similar to the previous researches of Doan and Oboh & Kushwaha (Akpan et al., 2013; Doan, 2015; Oboh & Kushwaha, 2009)). However, it was contrary to Ali and Khattak's study that had explained a small farm equals little profit, so to improve their production, the small farm owners need more credit for new farm technology and inputs (Ali & Khattak, 2011). The result of social capital had positive and significant ($p < 0.001$) impact on access to credit. Mwangi and Ouma supported this finding when they also found similar results (Akpan et al., 2013; Mwangi & Ouma, 2012).

Conclusions

This study was conducted to identify factor determining orange farm owners' access to credit in TuyenQuang Province. Data collected from a total of 107 orange farm's owners across the study area. From the literature review of access to credit and the local situation, the study used eight socio-economic dependent variables to assess the credit access of orange growers. The

logit regression model revealed that age, education level, orange cultivation experience, farm size, and social capital are the major factors which positive influencing on access to credit by orange farm's owners in the study area. Otherwise, the study also conducted that family size has negative relationship towards farmer access to credit. Social capital is the independent variable most influence access to credit by orange-farm owners in TuyenQuang Province (the coefficient B of 2.907 and the Exp(B) of 18.294). Based on the strong impact of this variable, the study recommended that orange farm owners in the study area should cooperate. Cooperative will ensure appropriate information sharing, risk reduction, and increase awareness of the matter relating to farm credit. In fact, cooperation had existed for a long time in Vietnam, but it revealed the weakness in organization that caused the diminution members. In recent years, cooperative model has reconstructed with a stronger link in agricultural production and consumption. Even so, the farmers still hesitate to join it. Local government should have a strong incentive mechanism for the cooperative to develop its strengths. In addition, the extension systems should be strengthened for more effective information dissemination on technical cultivation, farm management, and especially on credit issues. The provision of credit-related information could also change the risk attitude towards credit for those farmers who are not currently accessing credit. Vietnam Bank for agriculture and rural development and local authorities could coordinate to organize training courses for farmers to have more information on credit issues and easier access to credit.

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SMALL FAMILY FARMS IN INDONESIA: CHALLENGES AND INVESTMENT FOR FOOD SECURITY

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Abstract

Small family farms have been defined in many ways, from farm-land holder threshold to many other factors such as labor, asset, and resource. They are key for maintaining nutritional diversity with higher land productivity and diversity of production. They contribute to addressing key challenges related to equity, poverty and employment, such as better opportunities for civic and social engagement, more attachment to local culture and landscapes, and higher levels of trust within communities. In Indonesia, the average of small family farms land is 0.6 hectares, which then varies in each island. Half of the country's population is living in rural areas, where family farming is the predominant activity. In practice, they face some challenges, such as lack of appropriate technologies, difficult access to financial resources and market / distribution channels, demographic pressure impact on declining farmland, weak infrastructure, and environmental issues. Another fact is that in Indonesia 1/5 of small family farms live below national poverty line. Therefore, Indonesian government respond to this situation by promoting some investments for small family farms in order to increase food security in rural and food insecure communities. This paper is based on literature review related to small family farms, as well as extended review from field findings through observations and interviews. Qualitative approach has applied in collecting data. This paper aims to explain more about the characteristics, challenges faced by small family farms, and two investments for farmers in Sekayam sub-district, Sanggau district, which is located in the inter-country border area between Indonesia and Malaysia.

Keywords: *Small family farms, food security, Indonesia.*

Introduction

Small farms, also known as small family farms, have been defined in a variety of ways. The most commonly used criterion is land, for instance less than 2 hectares (Thapa, 2009) or below 5 ha (EU, 2011). From all thresholds, however, the appropriate size threshold must be adapted to regional and national situations. For example, the one and two ha thresholds are relevant in Asia, while a small Brazilian farm may measure up to 50 ha. In Latin America and in the Caribbean there are essentially two main categories of smallholders: first, those that rely significantly on non-farm sources of income, and second, those whose livelihood predominantly depends on the operation of their farms (HLPE, 2013). Others describe small farms as those depending on household members for most of the labour (Lowder et al., 2014; Brunori & Bartolini, 2016), subsistence orientation (Hazell et al., 2007), low asset base and operating below 2 hectares farmland (World Bank, 2003), and limited resource endowments (Dixon et al., 2003). While, other definitions bring family farming closer to that of a 'peasant farm', relating to ownership or tenure of land and means of production, to a strong component of household labour, to a relative autonomy from conventional markets and technologies (Ellis, 1993; Van der Ploeg, 2013) and to the concept of 'simple commodity production' (see, Friedmann, 1978).

According to FAO report (2018), one out of every nine people in the world are starving, and By 2050 the world's population will reach 9.1 billion, 34 percent higher than today, and the FAO is concerned that the world may not be able to produce enough food for the global population (FAO, 2009). Small family farms are key contributors for food security and nutrition, producing 90 % food around the world (HLPE, 2013). They are key for maintaining nutritional diversity (Herrero et al., 2017), with higher land productivity and diversity of production (FAO, 2014a, pp. 16-17; FAO and OECD, 2012; Larson et al., 2012; Wiggins, 2009; Lipton, 2006; Sen, 1966). They contribute to addressing key challenges related to equity, poverty and employment, such as better opportunities for civic and social engagement, more attachment to local culture and landscapes, and higher levels of trust within communities (Pretty and Bharucha, 2014; Donham et al., 2007; Lyson et al., 2001; Jackson-Smith and Gillespie, 2005). While large-scale industrial farming places the interests of local communities at risk (MacCannell, 1988; Lobao and Stofferahn, 2008; Lyson, 2004; Crowley and Roscigno, 2004). In addition, the positive spillover effects of family farming-generated growth on local rural non-farm sectors have been found to be especially strong (Ngqangweni, 1999; Bautista and Thomas, 1998), even shaping wider poverty reduction progress at the national level over the longer term. Moreover, as explained by HLPE (2013), enabling family farmers to fulfil the roles described above can impact on accelerator of progress across key elements of the Sustainable Development Goals (SDGs), such as contributing to end poverty (SDG 1), end hunger (SDG 2), ensure healthy lives (SDG 3), water management (SDG 6), terrestrial ecosystems (SDG 15), taking action on climate change (SDG 13), equitable growth, employment and equality goals (SDG 8 and SDG 10).
This paper aims to explain: 1) characteristics of small family farms in Indonesia; 2) the challenges faced by small family farms; 3) the investment for small family farms.

Methods

This paper is based on literature review related to small family farms in Indonesia, as well as an extended review from field findings through observations, FGD, and interviews. The research is conducted in Sekayam sub-district, which is located in the inter-country border between Indonesia and Malaysia. This area is part of Sanggau District, West Kalimantan province in Indonesia. Qualitative approach was applied in collecting data, with a total 44 informants, consist of 16 key informants, 15 participant farmers, 4 non-participant farmers and 9 informants for 3 focus group discussion (FGD) (farmers and other key informants). While the data collection technique used in this study are literature review and documentation, focus group discussion (FGD), in-depth interview, and observation. Data collection was conducted either directly or indirectly, from April until August of 2019.

Results and Discussion

In Indonesia, the majority of farmers are engaged in small family farming, which is generally carried out on modest plots averaging 0.6 hectares (FAO, 2018). Java island is predominant in the country's food production of crops with a farm size of about a quarter of a hectare or less per farm household. While in the outer islands like Sumatra, it is about one hectare or more for each farm household. Many native people in the outer islands own larger areas of land, which they have inherited from their ancestors (Septiani, 2015). In Kalimantan, they can have more than one hectare per household, because the population in Kalimantan is only 6.17% of total population of Indonesia, while the island size is more than 4 times bigger than Java island, which has 56.46% of total population (see table 1).

Table 1. The five largest islands in Indonesia and their population

No	Name of Island	Size (km^2)	Population	Share of population in Indonesia (%)
1	Kalimantan	544,150.07	16,209,800	6.17
2	Sumatra	480,793.30	54,168,100	20.44
3	Papua	421,991,20	4,260,000	1.61
4	Sulawesi	188,522.36	19,461,600	7.34
5	Java	129,438.28	149,635,600	56.46

Source: BPS, 2019

Table 2 below shows classification of farm size and number of farm households, where 58.73% of total farm household (16,257,430 households) hold below 0.5 hectare of farming-land. On the other hand, FAO report in 2018 stated that 93% of total farmer in Indonesia are smallholders (FAO, 2018). It means, the number of smallholder cover family farming holding a farming-land from 0 to 2.99 hectares. Hence, we simply can use this standard of "small" for family farming. Nonetheless, for native farmers who live in Kalimantan and Papua islands, they could have more than 3 hectares land per household, but still consider as small family farms.

Table 2. Number of Farm Households by Farm Size, 2018

Farm size (ha)	Number of Farms
< 0.50	16.257.430
0.50 – 0.99	4.498.332
1.00 – 1.99	3.905.819
2.00 – 2.99	1.627.602
3.00 – 3.99	607.908
4.00 – 4.99	323.695
5.00 – 9.99	374.272
≥ 10	87.059
Total	**27.682.117**

Source: Modified from BPS,2019

The farming activities are operated and managed by a family and predominantly rely on family labor, including both men and women and even their children. Half of the country's population is living in rural areas. Family farming is the predominant activity in these areas, not only providing food for the nation but being also important for the socio-economic, environmental and cultural roles of Indonesia (Septiani, 2015).

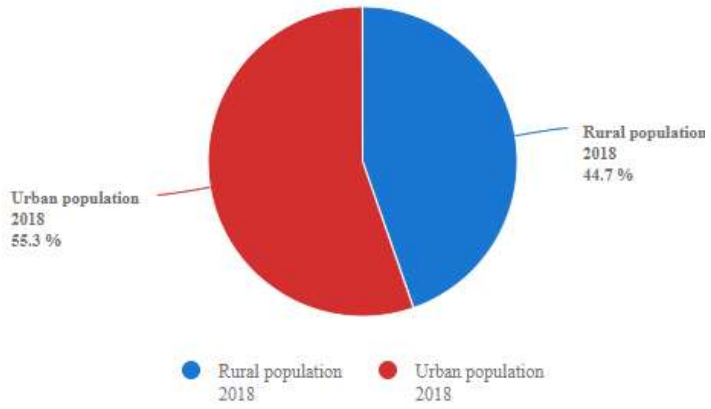


Figure 1. Percentage of national population (Source: FAOSTAT)

Based on the report of FAO (2018), on average, on-farm activities contribute only 49 per cent to the annual income, which is one of the lowest shares for smallholders in Asia. The

occurrence of shocks, such as a delay in monsoon rains which can harmfully impact on agricultural production, motivates small family farms to diversify their annual income with non-agricultural sources. Nonetheless, one-fifth of the family farms in Indonesia is still live below the national poverty line.

Small family farming plays a vital role in national food production, but at the same time, they face some difficulties. Slow implementation of technology penetration is one of them, only 10 per cent of the country's smallholders practices a high level of mechanization (FAO, 2018). Certain program to simplified access to credit schemes have enhanced the livelihood of the farmers. Nevertheless, only 17 percent of the farmers are beneficiaries with a credit that amounts to USD 420 annually. A high share of income spent on food and agricultural inputs limits the potential of smallholders to sustainably re-invest the amount of credit (FAO, 2018).

Declining farm sizes due to demographic pressure is also a main issue, which small farmers have to deal with, as population increases every year, and the need of housing also significantly increases. In many rural areas where the land is used for rice fields, now it is common to find housing areas built by developers without proper planning and, certainly, this land conversion has a direct impact on the low figures of rice production (Septiani, 2015). Furthermore, weak infrastructure such as poor access to roads and ports has become the main constraint in transporting agricultural products, which can make missing linkages between input and output markets. For instance, this problem will prevent vegetable products to be delivered in markets quickly, which means the limitation of the market. In harvest time, farmers face price volatility due to the amount of products that is not absorbable by the limited market. Moreover, the total number of islands in Indonesia is more than 17,000 according to the Indonesian Naval Hydro-Oceanographic office (ADB, 2015), which is uniquely challenging, especially to linkage food products between islands.

Environmental factors also contribute to the lower productivity in some areas and planting seasons. El Niño, a climate cycle in the Pacific Ocean with a global impact on weather patterns, has a significant impact in lowering crop production, especially that of rice. When the rainfall is high, the plants are attacked by fungi, or like leafy vegetables, whose leaves are damaged due to heavy rain. In some areas, where most agricultural land situated on the banks of river, populations have to deal with flood during monsoon season. The rainy season usually occurs two times a year, and every rainy season usually occurs for more than one month. Besides, long period of drought also caused crop failure. In many areas in certain seasons, crop failure also happened due to a wide array of pests and diseases, which is also associated with climate change. By accurate monitoring and mitigation, the outbreak of certain pests and diseases can sometimes be predicted, and at the same time by the use of tolerant or resistant cultivars. Hence, to deal with weed and pest attacks, the availability of herbicide and pesticide are important. If famers are not preventing pest attacks, they can fail to harvest. This, in turn, can decrease their ability to access farm input such as seeds and fertilizers.

The availability of fertilizers and seeds at accessible prices is also important for the sustainability of small family farming. Actually, the government has promoted subsidizing fertilizers and seeds programs, but some regulatory burdens affected the impact of these programs and even made them slow to be perceived by farmers. Low assessment of farmer needs is also another issue, that sometimes occurs when farmers receive farming input aid not in the best time. For example, there are farmers who received rice seed assistance when the rice planting season was over, so it cannot be used directly. Likewise, with fertilizers, aid comes after harvesting. To deal with all those challenges, investment has to be made for small family farms, and there should be strong commitment and effort from all sides at the same time. The government, industry, research institutions and the farming community itself, have to work together to achieve the goal of sustainable agriculture systems in Indonesia, which is

essential not only to produce enough food for the nation, but also to achieve food security in wider perspective.

Table 3. Smallholder Farmer Data Portrait in Indonesia

Small Family Farms in Indonesia		
Farm Aspects	Average farms size (ha) % of smallholders on total farmers % of female headed households	0.6 93 11.3
Income and Poverty	% of income from crops production % of income from on farm income % of income from agricultural wage labor % of income from non-agricultural activities and self-employment % of income from public and private transfer Smallholder poverty rate (national poverty line)	47 49 6 30 12 18
Capital & Input	% of household using motorized equipment	9.6
Constraints	% of credit beneficiary households Distance of household from road (km)	17 2.3

Source: Modified from FAO (2018).

One of investments released by the government was Kawasan Mandiri Pangan (KMP) program. The Indonesian Ministry of Agriculture has issued the Indonesia Minister of Agriculture Regulation Number 06/Permentan/OT.140/1/2014 about Desa Mandiri Pangan Guidelines 2014. Where the scope of *Desa Mandiri Pangan* activities includes *Kawasan Mandiri Pangan* (KMP). The general objective of the program is empowering the poor/food insecure to be self-reliant. The outputs are distribution of social assistance funds, and training/assistance for affinity groups. While the expected outcomes are to increase income and purchasing power of the people and increase access to food, in order to achieve food and nutrition security for the community.

On the other hand, the Ministry of Agriculture also implement another program, which is *Pengembangan Usaha Pangan Masyarakat*(PUPM) activities, or community food business development program. This program has executed based on Indonesian Minister of Agriculture Regulation Number 06/KPTS/KN.010/K/02/2016 about general guidelines for Community Food Business Development (PUPM) in 2016. The purposes of PUPM are: 1) to absorb national agricultural products at reasonable prices and benefit farmers, especially basic and strategic foodstuffs, such as rice; 2) support the stabilization of food supply and prices, and; 3) provide easy access for consumers/communities to basic and strategic foodstuffs at affordable and reasonable prices. This program was applied by supporting *Toko Tani Indonesia* (TTI), in order to realize the purpose of the program. The program simply aims to give the community in the border area more access to rice as a staple food, with affordable price, and to absorb their grain at the same time.

Conclusion

KPM program is an investment to simplified access to credit for farmers. As discuss above, thought access to credit have enhanced the livelihood of farmers, only 17 percent of the farmers are beneficiaries with a credit. While, PUPM program is an effort to provide wider market for farmers, as missing linkages between agricultural product input and output markets, or the limitation of the market still become a classic problem to be solved. Both of

the programs are then expected to support the realization of food security for local community.

Further work will then focus on these two investments for farmers in Sekayam sub-district, which is located in the inter-country border area between Indonesia and Malaysia. This area is part of Sanggau district, West Kalimantan province in Indonesia. It is an area of 841.01 km^2, with a total population of 34,488 inhabitants. There are three (3) communities, which are involved in the program, namely Ruis hamlet with a total population of 410 people, Kenaman hamlet with a total population of 1,370 people, and Berungkat hamlet with a total population of 1,018 people. There are three farmer groups in Ruis hamlet with a total of 78 farmers, 7 farmer groups in Kenaman hamlet with a total of 166 farmers, and 6 farmer groups in Berungkat hamlet with a total of 153 farmers. Our work will then assess how the KMP program impact on local food insecurity, and in particular, how it has affected food availability, food access, and utilization of households, with the aims: 1) to explore the implementation of the program in the research area; 2) to explain whether the program affects farmers' decisions of production, marketing, and consumption, and then; 3) to evaluate the impacts of the program through analysis of household food security of family farmers who were involve in the program, in three aspects, which are food availability, food accessibility, and food utilization. While for PUPM program, rice supply chain and local food security will be questioned, with the following overarching research questions: 1) how efficient is TTI in rice supply chain; 2) how is its impact on local food system, for the staple food (rice), and; 3) how does the program has been effective in addressing local food security in two aspects, which are food availability and food access.

This work not only brings a new approach to assess long-term outcomes (cf. impact) of the programs on population food security, but will also represent a context-specific intervention for the community, for further food policy-making and the achievement of food nutrition security of local people.

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TABLE GRAPES IN APULIA REGION AND INTERNET OF THINGS: INNOVATION AND SUSTAINABILITY. THE CASE OF THE IOF (INTERNET OF FOOD AND FARM) PROJECT

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Abstract

In a context of increasing economic competition, Italy is certainly the cradle of excellent agro-food products, recognized and appreciated at global level. Among them table grapes play a vital role in the panorama of Italian production, as witnessed by official data provided by ISTAT (National statistical institute) and related institutions. This is particularly true with regard to Apulia region, where the vast majority of production and cultivated fields are located. The need to concile quantity requests with traditional high quality and, as well, with sustainability makes it essential to employ effective ad hoc technological innovations. The adoption of Internet of Things (IoT) devices and, specifically, the creation of an IoT network to monitor the complete supply chain of table grapes, from field to fork, is of great importance and interest. Generally speaking, the mentioned devices and network allow farmers to monitor and optimize the growing behaviour of grapes and ripening, jointly with the amounts of fertilizer and pesticides used, as well as the irrigation process. All these features strongly rely on IoT devices and systems deployed on the field, in the packaging phase and in transportation. The present contribution aims at conducting an in depth analysis of the Internet of Food and Farm (IoF2020) project use case in Apulia region and its implications for the future enhancement of sustainable cultivation of table grapes.

Keywords: *Innovation, Internet of Things, Table grapes, Apulia region, Sustainability*.

Introduction

During last decades agriculture has experienced major changes, with technical developments playing a major role. Since the end of the Second World War, agrochemicals and mechanization participated in significantly increasing agricultural production at global level, despite a sharp drop in the rate of employment in the same sector. Nowadays, the digitalization of agriculture appears as the "natural continuation" of a mechanization process that has been underway for more than seventy years. It might be, therefore, more appropriate to approach this issue as a process that takes its roots throughout the last century, rather than considering it as a sort of revolution, a sudden and rapid break in technological equipment and a related profound transformation in the agricultural sector. In the past, as an example, farmers used to organized and standardize part of their activities, such as recording the level of precipitation and keeping track of it on calendars, or recording the doses of fertilizers on notebooks, thus creating first acts of digitalization of agriculture. Nowadays, this digitalization is developing on new, connected media, often considered as a breakthrough innovation for two main reasons: the development of electronics and robotics which, over the past thirty years, have introduced equipment such as computers and sensors on farms; the spread of communication and information sharing technologies, and related multiplexing that allows better sharing of information between devices. As a consequence, mere data collection

has given way to an exponential increase in the data collected, shared and processed (Pillaud, 2015; Laborde, 2012).

In line with the proposed approach, i.e. considering the digitalization of agriculture not as the advent of a "new era" capable of replacing the technical tools, the ways of communicating and being in touch, and, why not, the professionals, but, rather, as a transition part of a long professional history, it becomes vital to address the aspects and the effects of this digitalization in connection with the socio-cultural, economic and regulatory dynamics which, too, are contributing to contemporary changes in agriculture.

The sector at stake is, at a global level, currently going through a structural crisis despite the implementation of different agricultural policies. Farmers face the challenge of continuing to produce, but by controlling production costs, and limiting the impact of their activity on the environment. This triple concurrence of technical, economic and environmental performance is difficult to hold in a context of increased uncertainty, linked to technical tools and developments to implement, and risks, related, for example, to climate change or market instability. At the same time, it is possible to observe a constant decrease in the rate of employment in agriculture, accompanied by an increase in the importance, on one side, of improving productivity and working conditions and, on the other side, of meeting citizens and consumers demand for an evolution in their relationships with farmers and producers, so that their views are taken into account in the orientation of agriculture, for more transparency and traceability.

It is possible to argue that digitalization in agriculture is characterized by a sort of tension between different temporalities: the first regarding the long economic and social changes in agriculture and the relationships of citizens to food products; the second related to the faster technological innovations. The result is a scenario constantly evolving in light of new services and tools, considered as promises of responses to the economic, ecological and social challenges that have set up agriculture for several years in a context of crisis.

As far as the different aspects and reasons to adopt new technologies are concerned, it is possible to start from those related to the farm scale; at this level, digitalization supports the transformation of work in agriculture. Improving working conditions, both in terms of time saved and comfort, or increasing work productivity are among the first motivations for acquiring digital devices (Bethuel et al., 2017; Brossillon et al., 2015). This aspect stems mainly from a context where, on the one hand, labor productivity is constantly increasing and, on the other hand, farmers increasingly aspire to conquer more livable rhythms of life and work (Hervieu and Purseigle, 2013). Nevertheless, it is important to note how the adoption of digital devices is at the root of continuous debates among farmers. As an example, some producers invest in digital devices at the end of their career, with the idea that these devices make the profession more attractive for future farmers, while others denounce the replacement of human labor by machines, with a consequent decrease in the number of farmers. Furthermore, some farmers note that digital equipments allow them to increase their diagnostic capacity and make their decisions more reliable, while others question the future of the farmer's knowledge, as if the traditional expertise and sensitivity is under threat (Thareau & Daniel, 2019). These differences in judgments fully reflect the fact that digital devices can have ambivalent effects on work in agriculture (Hostiou et al., 2014).

From an economic perspective, the digitalization of activities can have an impact on the competitiveness of farms and, at the same time, lead them to consume fewer inputs. As to this aspect, the expected and possible gain in competitiveness depends mainly on the level of investments made and the modulation of variable costs achieved, thus making it necessary to consider market prices of the inputs and volumes saved. In general, the digitalization movement accentuates the long-term process of capital-labor substitution observed in agriculture; in addition, the development of connected objects allows a better control of

intermediate consumption and production costs. The tools related to the so called "precision farming" for crops (Thareau & Daniel, 2019) are part of this technical and economic scheme. In food processing companies, digital technology can optimize production processes, exactly as observed in other industrial sectors, and help to reconcile economic objectives of cost control and environmental sustainability.

Another important aspect to investigate is related to socio-digital media, in the perspective of a possible reconfiguration of collective dynamics in agriculture. As a matter of fact, although farmers are self-employed or work in small cooperatives (Courleux et al., 2017), they are part of dense social relationships capable of creating and structuring professional dynamics. These professional collectives, established or not, fulfill four main functions: to represent, to cooperate, to produce knowledge and to strengthen the links between farmers. In the actual scenario, these farmers' collectives are influenced by three main factors: firstly, it is possible to observe an increasing diversification in agricultural commercial structures, in line with different productive specializations and systems; secondly, in rural areas farmers are nowadays more dispersed and, as a result, their relationships are less and less close neighbor ones, networks extend spatially. Finally, farmers are led to interact with more diversified actors, whether these are local elected officials, neighbors, committed citizens or consumers. These three mentioned factors lead to a sociological context marked by a form of proliferation and destabilization of traditional structures of professional sociability, and a consequent development of the uses of the internet and socio-digital media as part of this unique context.

These new social tools appear as opportunities to strengthen the functions of representation, cooperation, solidarity and the production of knowledge traditionally played by informal networks or by professional organizations (SCAR AKIS, 2016). Moreover, they offer new opportunities for relationships capable of overcoming contemporary difficulties or of responding to new challenges, such as strengthening social ties made more difficult by geographic distance or facilitating the involvement of farmers in public issues (Thareau & Daniel, 2019).

At the end, the result is the strengthening of collective dynamics based on technical proximity rather than geographic one. These digital groups exchange publicly on forums, microblogs (Twitter) and formalize, by doing so, widely accessible technical knowledge. Spatial and temporal structuring of interactions changes, and it is possible to observe the formation of information ecosystems fed between peers, which publicize the diversity of technical approaches in agriculture (Daniel et al., 2019). Thus, these digital media appear as tools responding to contemporary challenges in the evolution of modes of interaction between farmers, and between them and third parties; furthermore, they allow the development of collectives outside the established framework of professional agricultural organizations (Le Guen, 2008).

Materials and Methods: The IoF project

The Internet of Food & Farm 2020 (IoF2020) project aims at investigating and fostering a large-scale implementation of Internet of Things (IoT) in the european farming and food sector, with the objective of bringing a paradigm shift in this domain, by drastically improving productivity and sustainability. The added value of smart webs of connected objects, that are context-sensitive and can be identified, sensed and controlled remotely in the agri-food sector is at the core of the project itself, composed by 5 trials, and 19 case studies (www.iof2020.eu). With its great potential, the project is in line with recent initiatives showing the eagerness of the sector to seize the opportunities offered by ICT, network and data–oriented technologies. However, current available applications are still fragmentary and mainly used by a small group of early-adopters; as a consequence, IoF2020 aims at providing

solutions and facilitating the large-scale uptake of IoT, by addressing the organizational and technological challenges the European farming and food sector faces today. In general, IoT can be used to optimise the quality of EU products while minimising possibilities for fraud, by enhancing transparency and traceability. This is particularly true with regard to the need to keep up with international competition, for both conventional and organic production; IoT technologies can be used to obtain higher quality and environmental sustainability along with decrease in production cost. Focusing on 19 use cases spread throughout Europe, the project provides solutions to 5 agri-food areas: arable farming, dairy, meat, vegetables and fruits and takes into account their own needs and obstacles. Moreover, IoF2020 involves all the stakeholders in the food chain: from farmers, cooperatives, equipment and logistic suppliers, food processing companies, to consumer organizations and it includes ICT developers, all with the objective of assessing and improving the technologies at stake, ensuring they meet the requirements and the expectations of the sector (Shinton, 2015).

As to the specific Table grapes Use Case, at the core of the present contribution, it is vital to note how table grapes are economically important products in the Mediterranean Europe. Indeed, the EU 27 is the third larger producer of table grapes in the world after China and Turkey. As a matter of fact, the value of the marketable yield of table grapes, more than any other crop, depends on the level of the quality standards (i.e. colour, berry size) achieved and imposed by consumers and supermarkets. Getting the quality level requested is not an easy task table grape growers have to accomplish, since the crop has a short shelf life, is highly sensitive to pests and diseases and can easily be damaged at any stage from harvesting, through storage and along the transportation process. Moreover, it is necessary to take into account the environmental restrictions imposed on farmers by supermarkets and environment agencies during production and post-harvest stage.

Therefore, there is a need for a modern production system whereby higher quality table grapes are effectively produced and delivered with minimum impact on the environment (Soulignac et al., 2019). As the product must reach the final consumer, efforts must cover the entire food chain from field to fork. Hence, the two major aspects are field production (agronomy and harvest) and post-harvesting (storage and logistics) taking into consideration the possible production methods constraints and the environmental impacts (i.e. water and carbon footprint). Generally speaking, some of the challenges faced by table grapes production at farm level can be summarized as follows: emerging pest and disease pressure encouraged by warmer climate, tolerance to chemicals and limited chemical options, especially within the organic farming; water wastage caused by inappropriate irrigation scheduling and/or inefficient water application systems with negative consequences on yield and quality; high labour costs and limited access to labour; manual picking, sorting and packing with a high probability of human error; maintaining consistent quality and maturity production output. Furthermore, the post-harvest challenges to be addressed in order to deliver good quality products to final consumers are:

- Efficient packaging and prolonged shelf-life systems;
- Maintaining the cold chain throughout the supply chain (storage and transport);
- Efficient storage methods to increase the shelf life of the product, reduce wastage, cut on production cost and carbon footprint;
- Traceability throughout the entire food chain to enhance food security.

Nowadays, technology is well developed and mature enough to build a robust IoT at an affordable cost. The main advantage of using technology in agriculture is that complex data can be automatically collected, elaborated and used to support farming practices to achieve a more profitable business with less impact on the environment, by cutting input losses and boosting quality and quantity. As mentioned above, regardless of the obvious beneficial effects of using IoT in agriculture, technology adoption has not reached a desired mass adoption level and is still limited to very few large agribusinesses.
As to potential barriers for technology adoption in the on-farm production (TRL8-9), in particular, and in the entire food supply chain in general, it is fundamental to cite:

- Equipment cost: building a network of devices embedded with electronics, software, sensors and network connectivity can be relatively expensive and beyond the economic capacity of small farmers;
- Complexity of the system and lack of skills from the end users side: even though proved to be beneficial, complex systems might not go beyond the research/experimental stage (TRL5) unless supported by tutorials, training courses and Graphical Users Interface (GUI) tools for end users;
- Social aspect: especially with the old generation farmers, that might struggle with the latest technologies and/or prefer to manage their own business in the traditional way they are used to;
- Absence of clear evidence of benefits: the potential benefit of using technology must be quantified and well documented if end users are to be convinced to change their business and invest in new equipment;
- Lack of appropriate infrastructure, especially in remote rural areas (i.e. electricity, internet and mobile network coverage).

Results and Discussion

The actors involved in the UC at the core of this contribution, all part of the value chain of table grape, gain potential benefits from the implementation of the mentioned technologies (Verdouw et al., 2016; Vermesan and Freiss, 2015). Farmers and their associations, IoT developers and suppliers, research centres and their spin-off, local stakeholders and consumer associations and, finally, the logistic organisation, they all decide to create a virtuous network capable of giving rise to positive synergies.
The IoT resources implemented refer in particular to: at farm level, a certain number of devices and/or tools installed/used in order to measure all the parameters (sensors, robots, drones, measuring tools, and so on); in the post-harvest phase, some "smart label" or modified atmosphere packaging.
As a matter of fact, farmers are the first to benefit from the technology at stake, as it helps them to improve their farming practices, effectively use their water input and protect their crop and finally produce more marketable yield. Technology companies also take advantage to expand their ICT market into agriculture production and post-harvest. Improving packaging and post harvesting techniques, longer shelf life and hence less crop wastage in storage and on the supermarket shelves constitute important aspects to take into account; the entire society also benefits, since water wastage is reduced with IMIS tool and the pesticides are more effectively used with positive impact on human health and on the environment.
As mentioned above, the main objective of the table grapes UC is to develop an IoT adapted to the supply chain of table grape with the main focus on the on-farm production (agronomy and harvest) and post-harvesting (storage and logistics) system. At field production level, the aim of the proposed IoT is to improve water usage through a quality oriented irrigation scheduling and develop a decision support system for an effective pest and disease control by

extracting and analysing weather forecast data. A web benchmarking platform is developed to identify best practices, gaps in the farming business performance and accordingly draw an action plan for a continuous improvement in the table grape farming.
At post-harvest level, IoT are applied on table grape to improve the packaging process, storage and transport in order to collect all the information necessary to be informed in real time about the shel life of the packaged product.
As to the italian UC, in the context of the IoF project, all the mentioned challenges and aspects are implied. Specifically, the use of Blueleaf, created by Sysman Srl, a device capable of managing irrigation, water consumption and pests control is underway, together with the Blow device. This last, of particular interest, is the registered name of the device used for controlling gaseous exchanges between the inside and outside of a container for solid food products. The device comprises a main body applicable to the container so the gas exchange does not depend on the type or material of the container. Blow allows triggering of gas exchange in both the directions through the device; easy to use with packaging, it modulates the gas exchange to help overcome specific problems during storage of table grape. The sensor connected for all the packaging with a cloud can give an exactly measurement of gas exchange and shelf-life measure (Figure 1).
It is, therefore, possible to observe how the implementation of these analysed devices can effectively improve the efficiency of companies performance, and lead them to a real sustainable development path.



Figure 1: Table grapes (Scarlotta Seedless) packed with Blow
Source: IoF Table grapes UC – CIHEAM Bari

Conclusion

As to expected results, in view of the project ongoing, they refer to the following aspects:

- Decision support tool for appropriate irrigation management to enhance yield and quality and reduce water and energy wastage;

- Intelligent water application system that can be programmed to fully automate irrigation or to apply variable rate irrigation depending on the variation in the crop water needs;
- Alert system in real time for pest and disease incidence based on current and future weather forecasts;
- Benchmarking web platform that collects data from growers and provide them with a score card on their performances, with recommendations in order to reach the identified best practice.

The IoT developed for the table grape supply chain can easily be implemented for other crops and in different locations. Also the technologies analysed for the italian UC is different from the greek one, and hence there is a big opportunity to integrate the technology of both UCs for the same scope.

Moreover, the possibility to broaden the application of the analysed and described technologies to other regions, especially in the Mediterranean area, constitutes a a fundamental tool for sustainable development, in all its aspects (social, economical and environmental).

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PROBLEMS, PROSPECTS AND EXPERIENCE IN THE IMPLEMENTATION OF PRECISION FARMING IN THE REPUBLIC OF BELARUS IN THE CONTEXT OF NATIONAL LAND USE

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Abstract

Belarus has a high potential for introducing precision farming systems in agricultural production. One advantage is the availability of more than 1380 agricultural enterprises, with an average area of agricultural land of 5.3 thousand hectares, and an average area of arable land of 3.5 thousand hectares. Of these agricultural lands 2.55 % are in a private land use, while 97.45 % are state-owned agricultural enterprises. Furthermore, agricultural land can only be owned by the state. Based on the current situation, the development of precision farming systems should focus on large agricultural enterprises, and not private farms. However, there are the widespread introduction of precision farming systems in the country's agricultural industries. Most important is the current system of on-farm land management that is focused on traditional energy and resource-intensive farming. In the framework of this research for the introduction of precision farming, the following results were obtained: 1) The most effective methods for creating task maps for the differential application of mineral fertilizers were identified; 2) a technique for using ultra-high resolution remote sensing data to predict the yield of grain and forage crops was developed. Implementation of these measures when introducing differential fertilizer application allows increasing the profitability by: 2.2 % for winter crops, 1.3 % for sugar beets, 1.1 % for rapeseed for oilseeds and 0.8 % for malting barley.

Keywords: *Agricultural enterprises, Land management, Farming system, Profitability.*

Introduction

Successful socio-economic development of any state is a result of the complex interaction of three components - economic, environmental and social, which form a triumvirate that ensures sustainable development of its territory. The 2030 Agenda for Sustainable Development, which identified the 17 Sustainable Development Goals, was approved at the 70th session of the UN General Assembly in 2015. (Transforming our world… 2019).

The Republic of Belarus was active in the development of this document at all stages and made commitments to achieve sustainable development goals aimed at increasing the level and quality of people's standard of living (Sustainable development…, 2019). Undoubtedly, a powerful and modern agro-industrial complex is an effective tool for ensuring the economic well-being and food security of the country. At the same time, land characteristics determine the production potential of the agricultural industry in general and farming in particular. In other words, both the economic efficiency of land use and the efficiency of agriculture as a whole largely depend on the quality of the land. (Myslyva *et al.*, 2017, 2018). Improving and developing the technical, technological, environmental and social aspects of land use to ensure the effective management of public relations for the use of land resources provides the high competitiveness of the national economy at the global level (Ibatullin *et al.*, 2012).

Land management plays a significant role in this process and provides for the organization of the environmentally and economically feasible use of land through the system of engineering,

technical, economic and legal measures, as well as to provide the efficient organization of the territory and the location of production. Intensification of the globalization processes of the world economy has led to a situation where the agricultural production of all countries without exception, regardless of the level of their economic development, has encountered a number of serious problems. The main ones are associated with both the increasing demand for non-renewable energy resources, fresh water shortage, and with the acceleration of urbanization processes and the reduction of areas suitable for growing crops, accompanied by increased erosion processes and desertification caused by global warming. In particular, over the past decade, a decrease in the area of arable land in Europe, according to the forecast estimates, will reach 1.12% by 2030 (Zarco-Tejada *et al.*, 2014; Daheim *et al.*, 2016; EU agricultural outlook…, 2018), while for Belarus this indicator ranges from 0.1% to 0.4% (Agriculture of the Republic of Belarus…, 2019).

Given the constant rise in the cost of energy resources and raw materials for the production of mineral fertilizers, as well as shortage of organic fertilizers, identifying ways of increasing the economic efficiency of land use is becoming urgent. The introduction of precision farming as a modern concept of agricultural management using digital methods to monitor and optimize agricultural production processes is one of the methods for its successful solution (Doerge, 1999), or mitigation of the associated problems.

The purpose of this study was twofold: 1) To review the prospects and existing problems in the implementation of precision farming technology or its particular elements in Belarus agriculture under the existing conditions of state ownership of agricultural lands; 2) to conduct an analysis of the results obtained when introducing elements of precision farming system in agricultural enterprises of the Mogilev region of the Republic of Belarus.

Material and Methods

Methods that were used in this research include analysis and synthesis method, a systematic approach method, abstraction, geostatistical and comparative research.

The studies were carried out in 2017-2019 on the territory of Gorky district of Mogilev region (Republic of Belarus) within the land use of RUE "Uchkhoz BGSHA" on an area of 8342.1 thousand hectares. The shape file with the placement of land within the study territory was created based on the results of digitization of planning and cartographic materials, which was obtained from the agrochemical survey of the territory of RUP "Uchkhoz BGSHA", executed in 2018 by the Mogilev Regional Design and Exploration Station of Agrochemicalization.

Identification of management zones for off-line differentiated application of mineral fertilizers and calculation of their areas within the study land use study were carried out using the functionality of ArcGIS version 10.3. The possibility of using ultra-high resolution remote sensing data to predict the productivity of feed and grain crops was assessed using the Phantom-4ProV 2.0 and Agisoft PhotoScan version 1.5.2. Vegetation indices (Red-Green-Blue Vegetation Index (RGBVI), Green Leaf Index (GLI), Visible Atmospherically Resistant Index (VARI), and Normalized Green Red Difference Index NGRDI) were calculated using QGIS software version 3.10.

Statistical processing of experimental data and the creation of mathematical models were carried out using Statistica version 12.0.

Results and Discussion

The European Parliament's report on Precision agriculture and the future of farming in Europe defines precision agriculture as: "an integrated information- and production-based farming system that is designed to increase long term, site-specific and whole farm production

efficiency, productivity and profitability while minimizing unintended impacts on wildlife and the environment" (Zarco-Tejada *et al.*, 2014). Optimization of all production processes is the key point and driving force of precision agriculture, the result of which is the rational use of resources, cost savings and reduction of negative environmental impact. Belarus has a sufficiently high potential for introducing a precision farming system or its individual elements in agricultural production. Among its main advantages is the existence of over 1380 agricultural enterprises with an average land use of more than 5.3 thousand hectares of agricultural land and over 3.5 thousand hectares of arable land as well as the concentration of agricultural land mainly in the state ownership (87.6% of the total area) (Agriculture of the Republic of Belarus…, 2019). In addition, a positive factor, which should be taken into consideration, is the concentration of agricultural land mainly in the state ownership (87.6% of the total area) (Register of land resources…, 2019). This opens up for agricultural producers the opportunities to receive government financial support for the implementation of precision farming systems, in particular for the modernization of technological processes and the purchase of high-precision equipment. A significant advantage is that Belarus has highly developed agricultural machinery and manufactures its own combines and tractors equipped with Trimble precision GPS positioning systems, which significantly reduces the cost of purchasing precision machinery for agricultural producers. Examples of this technique are the Palesse GS2124 combine harvester manufactured by Gomselmash OJSC, equipped with a yield mapping system and a tractor manufactured by Minsk Tractor Plant OJSC, equipped with the Trimble Autopilot auto-driving system.

It should be noted that according to the Land Code of the Republic of Belarus, there are two forms of ownership of land and land parcels - state and private; moreover, agricultural land is not private property and can only belong to the state. However, the Land Code provides for citizens the inherited lifetime tenure as an alternative to private property. There are 1,357 agricultural organizations and 2,652 peasant (farmer) enterprises operating in the republic. However, only 2.55% of agricultural land is owned by peasant farms, while 97.45% is owned by agricultural enterprises of various forms of ownership that are land users or tenants, but not landowners (Figure 1).

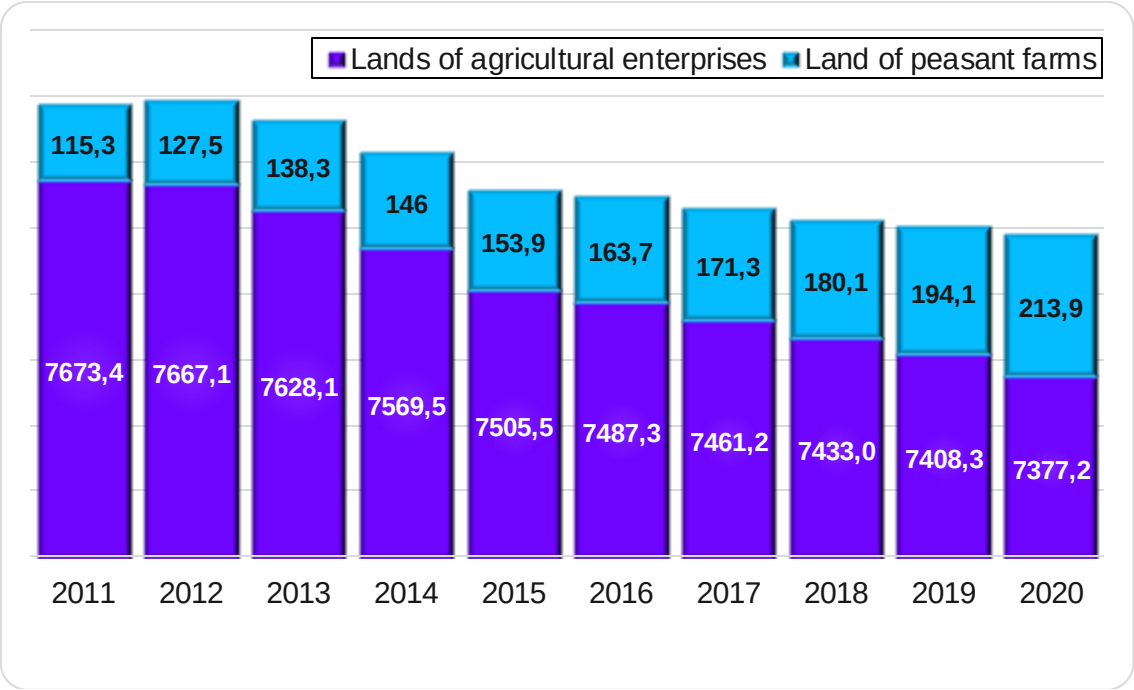


Figure 1. Distribution of agricultural land by land user categories, theous. hectares

Based on the current situation in the sphere of land ownership, the development of precision farming systems in Belarus can be carried out in large agricultural enterprises, and not in private farms, as is common in Europe and the USA.

Nevertheless, along with the advantages, there are both objective and subjective reasons that impede the widespread implementation of precision farming systems in the country's agricultural production. One of them is the existing system of on-farm land management, focused on traditional energy and resource-intensive farming, and not taking into account the presence of heterogeneities within a single field or land parcel - key factors for precision farming. It should be noted that on-farm land management is an element of land management that has been preserved in the Republic of Belarus since the USSR because of the preservation of both state ownership of agricultural land and agricultural enterprises that existed in the Soviet period in the form of collective farms and state farms. However, currently land management related to inter-farm land management is dominated in Belarus. These works consist mainly in updating the data of the land information system, developing draft land allotments, preparing technical documentation and establishing the boundaries of land plots on the ground in connection with the formation of agricultural and non-agricultural land uses.

At the same time, it is necessary to note the exceptional importance of conducting on-farm land management in the context of the transition to digital farming, because this particular type of land management creates the territorial basis for the efficient use of land, its protection and the introduction of advanced management systems (Table 1).

Table 1. Functions of on-farm land management in the implementation of elements of the precision farming system in Belarus

Precision farming system element	*Function of on-farm land management*	*The degree of implementation of the function*
Digital contours of crop rotation fields	Creation of vector models and a database of geospatial data on field boundaries	Partially implemented
Monitoring of intra-field variegation of soil fertility	Creation of a geo-positioned network of monitoring observations	Not implemented
Digital mapping of intra-field variegation of soil fertility	Creating predictive models of the spatial distribution of agrochemical, physicochemical and agrophysical soil properties	Not implemented
Digital yield mapping	Creating digital maps of the dynamics of intra-field crop productivity	Not implemented
Creation of task maps for the differential application of mineral fertilizers	Identification and delineation of zones homogeneous in the agrophysical and agrochemical properties of soils and agrotechnological characteristics of land parcels	Not implemented

The most important condition for the effective implementation of precision farming is the creation of relevant management-zone maps, which reflect the real state of land quality and make it possible to differentiate the application of mineral fertilizers and chemical reclamants, while maximizing the potential of the soil. In this regard, the main task of land management in the current socio-economic conditions of Belarus in the context of the introduction of precision farming is the development of a methodology for the creation of appropriate cartographic materials and differentiation of land use by the totality of land quality indicators. It is also extremely important to create geospatial databases on the available indicators of the quantitative and qualitative state of agricultural soils. According to analysts' forecasts, the market for such products in the structure of elements of the precision farming system in the EU countries will grow by 12% annually, showing a steady upward trend (Daheim *et al.*, 2016). The global market size of smart agriculture is expected to grow from approximately 9.58 billion U.S. dollars in 2017 to 23.14 billion U.S. dollars by 2022 (Statista, 2019). The

maximum market growth in the last decade is observed in countries such as the USA, Germany, Great Britain, France and other highly developed countries (Maloku, 2020).
Important and interrelated components of the registering and reacting precision farming technologies are the identification of management zones and their use for the differential application of phosphorus and potassium mineral fertilizers. It should be noted that management zones mean field subregions, which are determined by the relative uniformity of productivity of crops grown within its boundaries and/or soil parameters, and requiring the application of the same fertilizer rate, dose of pesticides, etc. (Doerge, 1999; Edge, 2019).
On the example of land use with an area of more than 8 thousand hectares, a methodology for the formation of homogeneous territorial management zones during on-farm land management with the introduction of precision farming was developed.
The management zone identification algorithm provides: exploration geostatistical analysis; determination of the required number of gradations of land quality; assessment of data clustering and analysis, search for data outliers; construction of interpolated rasters for a specific set of soil parameters; reclassification of rasters and multivariate analysis; converting the final raster into vector layers and determining the areas of the selected zones. As input parameters, it is recommended to use the data of agrochemical soil survey performed centrally every 4 years.
The strategy of differential fertilizer application, taking into account, in particular, national characteristics of the right of ownership of agricultural land, is the most acceptable in modern socio-economic conditions prevailing in Belarus. This strategy is based on fertilizing in the management zones in such a way that the quantity and ratio of nutrients, taking into account their soil reserves, is sufficient to obtain the planned crop yield (Figure 2).



- **STRATEGY 1:** Fertilizers are applied to management zones in such a way that the quantity and ratio of nutrients, taking into account their soil reserves, is sufficient to obtain the planned yield
- **STRATEGY 2:** Fertilizers are applied in such a way as to obtain the planned yield of a specific crop at a specific field with minimal doses
- **STRATEGY 3:** Fertilizers are applied in such a way as to obtain the maximum yield of a particular crop on a specific field with a limited amount of fertilizer

Figure 2. Differentiated fertilizer strategies

Within the individual crop rotation fields on the area of 1411.76 ha, it is possible to save from 2.5 to 21.8 kg/ha of the active substance of phosphorus and from 0.9 to 26.7 kg/ha of the active substance of potassium due to the redistribution of the dose of fertilizers for the planned crop yield taking into account the identified management zones. The maximum saving of phosphorus fertilizers is achieved when applying them for winter wheat, corn for silage and peas grown for grain while potash fertilizers provide maximum saving when applying them for winter wheat, sugar beets and spring triticale. The differentiated use of mineral fertilizers by reducing the cost of their purchase and use makes it possible to increase the profitability of growing winter grains by 2.2%, sugar beets by 1.3%, rapeseed for oilseeds by 1.1%, and malting barley by 0.8%.
The use of unmanned aerial vehicles in agriculture in the implementation of precision farming tasks is an innovation for the Republic of Belarus. A promising area that is just beginning to

develop in Belarus is the use of ultra-high resolution remote sensing data obtained from UAVs for monitoring and forecasting the productivity of fodder and grain crops. However, the use of agricultural drones has not found wide application for a number of reasons of an objective and subjective nature (Figure 3).

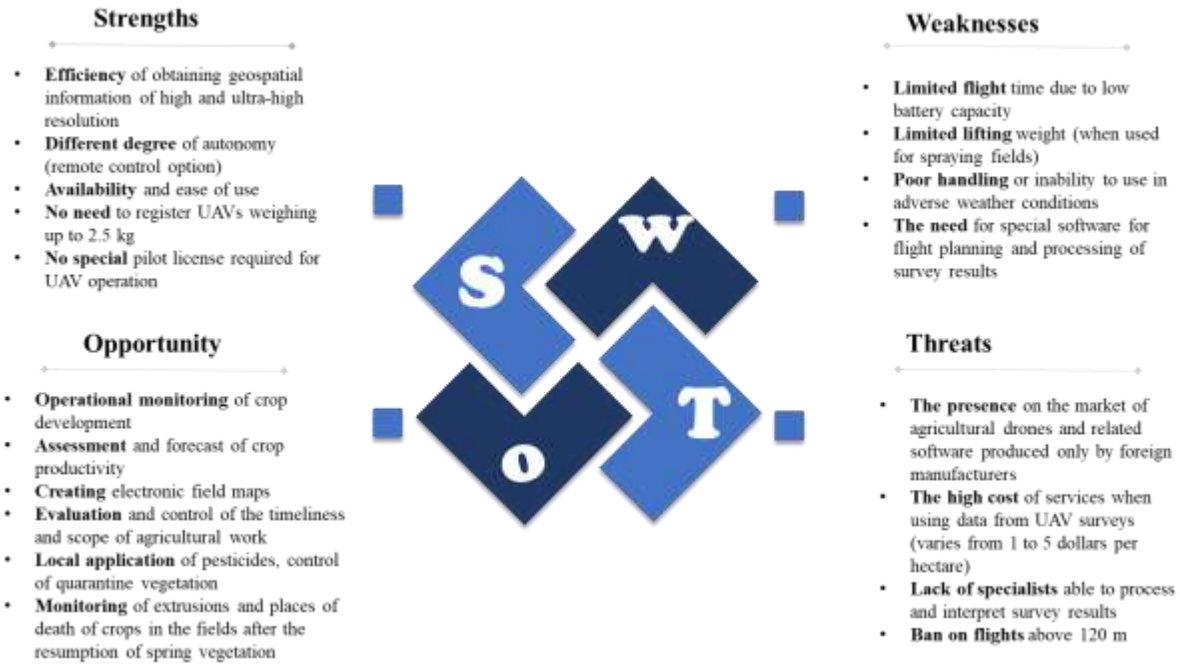


Figure 3. SWOT analysis of UAV use in agriculture of the Republic of Belarus (developed based on the results of author's own research)

Nevertheless, this technique is gradually being introduced into agricultural production, primarily in conjunction with the introduction of elements of the precision farming system. It was found that the data obtained from the quadcopter survey in RGB mode are excellent for determining the height of the *Silphium perfoliatum* and *Zea mays* from the model of the vegetation cover surface with an ultrahigh resolution of 2.5 cm in the field. Plant height obtained from surface models based on UAV survey data is a reliable indicator for assessing biomass productivity, since the correlation coefficient between actual and forecasted productivity values was 0.98 and 0.97, respectively. Vegetation indices RGBVI, VARI, GLI and NGRDI can also be successfully used to monitor and evaluate biomass productivity, and the most informative of them is the RGBVI index. However, long-term field research is necessary to ensure the reliability of predictive productivity models.

Conclusions

In order to ensure energy savings and the introduction of modern agricultural production systems in Belarus, the widespread introduction of the precision farming system or its individual elements is necessary. Among the factors contributing to the widespread adoption of precision farming in the country's agricultural production are more than 1380 agricultural enterprises with an average land use of more than 5.3 thousand hectares with state support and highly developed agricultural engineering resources.

Impeding the widespread implementation of precision farming is the existing system of on-farm land management, focused on traditional energy and resource-intensive farming, which does not take into account the heterogeneities within a single field or land parcel. Since agricultural lands in Belarus are the exclusive property of the state, the introduction of land use precision farming is impossible without digital land management. In this regard, the

primary function of modern on-farm land management is the identification and georeferencing of soils homogeneous in agrophysical and agrochemical properties and agrotechnological characteristics of arable land parcels in order to optimize agricultural land use while considering the needs of highly profitable crops.

The most promising elements of precision farming technology for implementation in Belarus are the off-line differentiated application of mineral fertilizers and chemical ameliorants, as well as the use of ultra-high resolution remote sensing data to monitor crop development and productivity forecast.

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GASTRONOMIC TOURISM FOR SUSTAINABLE DEVELOPMENT OF RURAL AREAS OF LATVIA

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Abstract

Sustainable development strategy of Latvia states that rural areas of Latvia are experiencing socio-economic depletion, therefore important long-term challenges have been identified. They are related to the creation of quality living and working spaces in rural areas, preservation of cultural heritage, diversification of economic development. By developing gastronomic tourism in rural areas, the socio-economic growth of rural areas can be promoted. Tourism offers that include gastronomic services are popular and in demand among both local and foreign tourists. Unfortunately, some gastronomic tourism service providers in rural areas of Latvia lack knowledge and information on how to create and successfully manage sustainable gastronomic tourism services. The aim of this research is to identify how gastronomic tourism can be used for sustainable development of rural areas of Latvia. Tasks to achieve the aim is to (1) to evaluate gastronomic tourism as one of the instruments of sustainable development in rural areas, (2) to determinate involved parties in development in rural areas, (3) to identify activities, that can be used for the sustainable development of rural areas. Following research methods are used: monographic, analysis, synthesis, data grouping. The research concluded that local gastronomy can be used for sustainable development of rural areas. Gastronomic tourism can be developed via efficient use of rural resources, formation of cooperation clusters, and by diversification, combination and re-profiling of rural farming services. Cooperation between involved parties is an important aspect for sustainable development of rural areas.

Keywords: *gastronomic tourism, sustainable development, rural development*

Introduction

Latvia, as a tourist destination, is becoming more and more popular every year. Data from the Central Statistical Bureau of Latvia indicates that during the last ten years the number of visitors served in hotels and other tourist accommodations has been increasing, as well as the number of foreign visitors in Latvia is increasing year by year (Central Statistical Bureau, 2019). Taking into account the positive trend of tourism growth in Latvia, need for observing the principles of sustainable tourism management is becoming more important.

Tourism offers that include gastronomic experiences are popular among both - local and foreign tourists. Recently, more and more food festivals, fairs, master classes with chefs, local food farms, organic food farms that provide tourism attractions are becoming more popular. Gastronomic tourist locations are in demand in various excursion programs and highly attended. The restaurants' efforts to include local and seasonal products and local and traditional foods in the menus are appreciated by local and foreign visitors.

High popularity of gastronomic tourism attractions can be used as an advantageous tool for the sustainable development of rural areas.

Unfortunately, some of the gastronomic tourism service providers in rural areas of Latvia lack knowledge and information on how to create and successfully manage sustainable gastronomic tourism products and services. Often, they are small businesses or home-based producers, that lack knowledge and experience on how to present their services to customers (story, presentation, dishes, interior, etc.), how to work with foreign visitors, how to promote their product, how to manage seasonality in tourism, what are the most effective cooperation models in particular destination, or how to coordinate and communicate their overall gastronomic tourism activities. The aim of this research is to identify how gastronomic tourism can be used for the sustainable development of rural areas of Latvia.

Materials and Methods

This paper is based on a literature review and discussion on published information in gastronomic tourism and rural development subject area. The focus of the literature review is to summarize and synthesize the arguments of the research topic. Tasks to achieve the aim is to (1) to evaluate gastronomic tourism as one of the instruments of sustainable development in rural areas, (2) to determinate involved parties in development in rural areas, (3) to identify activities, that can be used for the sustainable development of rural areas. Nacional policy planning documents of Latvia, regulatory enactments, and institutional framework, which directly and indirectly are related to the sustainable development of gastronomic tourism in rural areas of Latvia, are analysed. Official statistical data from the Central Statistical Bureau of Latvia are reviewed. The following research methods are used: monographic, analysis, synthesis, data grouping.

Results and Discussion

Gastronomic tourism which supports regional development by setting the link between food and beverages and tourism and strengthens local identity and culture as well as financial conditions should be more active and maintained. So, this will also contribute to protecting historical and cultural heritages and handing down them to the next generations and ensuring its permanence (Sormaza, 2016). However, gastronomic tourism is not defined as a separate type of tourism within the framework of the Latvian law, as it is considered as one of the types of cultural tourism. World Food Travel Association defines food tourism as follows: "Food tourism is the act of traveling for a taste of place in order to get a sense of place." Gastronomic tourism includes visiting food producers, eating festivals, restaurants and special places related to some special foods together with tasting a special dish, observing its production and preparation processes or eating a special dish from the hands of a very famous chef as well as seeing how a certain dish is being prepared. The industry of gastronomic tourism, which does not consist only of food guides and restaurants, covers any kind of culinary experience (Sormaza, 2016). However, looking to the gastronomic tourism in the rural areas, the greatest emphasis should be on sustainability, local authenticity, cultural heritage, local natural resources, and seasonality. Interest in local produce may serve to stimulate local awareness and interest and encourage community pride and reinforcement of local identity and culture. The development of strong local food identities and sustainable food systems in rural areas have substantial potential to grow, with tourism playing a significant role in this process (Hall, 2003). Rural Tourism Association of Latvia stresses that it takes at least 3 requirements to promote a good gastronomic tourism product in rural areas: (1) the food products must be local, good quality and well known within the region; (2) hosts themselves must be welcoming, enthusiastic and believe that local cuisine is special; (3) hosts

should provide a skilful presentation of food - explanation, story, design, table presentation, and appropriate price (Rural Tourism Association, 2011).

Gastronomy can also be a major conduit for tourists to appreciate the local culture of a destination and one of the primary motivations to visit a destination (Richard and Chang, 2018). Berbel-Pineda argue that gastronomy is in third place among the motivators for which tourists decide to visit a destination after cultural interest and nature (Berbel-Pineda, 2019). Rural Tourism Association of Latvia points that there are two main reasons for potential of gastronomic tourism development in rural areas of Latvia: (1) growing interest in a healthy lifestyle and organic clean food, often associated with small farms and local products, (2) the tourists` interest in the authentic and unique offer within different regions (Rural Tourism Association, 2011). Hjalager and Richards (2002) argue that authenticity in gastronomic tourism is increasingly important and will be more and more backed up with quality assurance schemes based on geographical areas, systems of production or other aspects of culture (Hjalager, Richards., 2002). Gastronomic tourism is mainly an experience. A direct experience in the production territory and the sensory and cultural experiences that gastronomy offers are new elements that can be added to the discovery of new and unknown landscapes and territories by tourists (Vázquez de la Torre, 2016).

Gastronomic experiences during travels are enjoyable and entertaining for travellers. However more additional benefits can be provided during gastronomic experiences. Positive economic impact, cross cultural connections, protecting and preserving local heritage and traditions, and contributing to the sustainability of destination and their culture. Gastronomic tourism contributes to economic, sociocultural and environmental sustainability (Stone and Migacz, 2019). Kivela and Crotts argue that regional gastronomic tourism positively influence regional competition, provides sales increase potential and provides cooperative marketing and cross-marketing opportunities for regional tourism destinations. (Kivela and Crotts, 2006) World Food Travel Association emphasizes that local governments should develop local food tourism strategies, ease regulations for home-based and small craft businesses, and align various food tourism providers to the same goals (Stone and Migacz, 2019). Tourism clusters have become popular concept of local and regional development and can increase regional competitiveness (Santos Estêvão, 2009).

In the rural regions of Latvia, gastronomic tourism projects have already been implemented, as well as some new started. Vidzeme planning region has succeed with such project as the title "European Region of Gastronomy" in 2017. The European Region of Gastronomy platform is raising awareness about the importance of cultural and food uniqueness, stimulating creativity and gastronomic innovation, educating for better nutrition, improving sustainable tourism standards, highlighting distinctive food cultures and strengthening community well-being. Latvia and Estonia in cooperation have launched gastronomic tourism project "Flavours of Livonia", which unites more than 200 small entrepreneurs, farmers, fishermen and producers. Rural Tourism Association of Latvia has developed several gastronomic tourism routes and brought together service providers and manufacturers that provide gastronomic tourism in rural regions of Latvia.

Continuing the development of gastronomic tourism, it is necessary to think about the creation of sustainable tourism products, formation of partnerships, organization of communication and coordination of other development-promoting activities. Different types of activities are regarded as supporting the aim of developing higher value-added gastronomic tourism products. Hjaleger and Richards emphasize some of them: (1) including gastronomic aspects in promotional materials of the region; (2) campaigns for particular products, connected to a region or season; (3) creating tourism appeal for existing food fairs and events; (4) reinventing, modernizing and commodifying historical food traditions; (5) establishing and marketing routes (Hjalager, Richards, 2002).

Legal framework and government involvement can play an important role in development of gastronomic tourism. Sustainable Development Strategy of Latvia until 2030 is the hierarchically highest national development planning document. This document states long-term development goals, priorities and territorial development perspective of the country. Rural areas of Latvia are experiencing socio-economic depletion, therefore important long-term challenges have been identified. They are related to the creation of quality living and working spaces in rural areas, preservation of cultural heritage, diversification of economic development. Surrounding cities should support rural areas, provide sales for agricultural products, workplaces and various services for the local residents of rural areas of Latvia. At the same time the rural areas, besides the production of agricultural and forestry products, should provide recreational opportunities for urban residents, good quality living space, as well as opportunities for non-agricultural businesses in the area (Sustainable Development Startegy of Latvia until 2030, 2010).

The competence of local governments in the field of tourism is related to the development planning of the territory. Local governments should determinate the perspectives of tourism development according to territory planning documents, provide support for development of new tourism services, as well as involve in maintaining existing tourism products and services.

By developing rural tourism and other alternative activities, including gastronomic tourism in rural homesteads or holiday houses, the preservation of rural areas and Latvian historical culture and traditions could be promoted. Rural tourism as *"a type of tourism in rural areas, which aims to provide the opportunity for tourists to relax, based on local social, cultural and natural resources, or the use of tourist accommodation in rural areas"* is defined in Tourism Law of Latvia. Rural tourism consists of cultural heritage that exists in rural areas; eco-tourism, nature-based tourism, where the main purpose is to visit natural areas, enjoy scenery, including plant and animal wildlife; and-tourism, which refers to the act of visiting farms for enjoyment of farm activities and education (Aytug, Mikaleili, 2017). Gastronomic tourism products in rural areas of Latvia should be developed according to rural tourism goals of Latvia.

Latvian Tourism Development Guidelines for 2014-2020 is the main tourism policy document in Latvia. The overall goal of this document is to ensure sustainable growth of the Latvia`s tourism sector by facilitating competitiveness of Latvian tourism services in export markets. Latvian tourism policy aims to increase the competitiveness of Latvian tourism supply that: (1) meets the criteria of sustainable tourism product development; (2) encourages international competitiveness, (3) reduces seasonal imbalance in tourism flows, (3) extends the average length of stay (Latvian Tourism Development Guidelines for 2014-2020, 2014).

Looking more specifically at the Guidelines, it states, that cultural heritage, including gastronomy, is an important resource in the development of tourist destinations. In addition, the recommendations in the Guidelines points to the need for development of *slow-food* tourism (use of local, natural and seasonal food products), gourmet tourism, innovative gastronomy, and promotion of the preparation and presentation of national food in a modern, tourist-friendly way (Latvian Tourism Development Guidelines for 2014-2020, 2014).

When studying the development opportunities of gastronomic tourism in the rural regions of Latvia, it is important to take into account the tendencies, challenges, development directions and solutions for sustainable rural development and sustainable tourism development specified in the policy planning documents: Sustainable Development Startegy of Latvia until 2030 and Latvian Tourism Development Guidelines for 2014-2020.

Conclusions

The overall goal of developing gastronomic tourism in rural areas of Latvia is to maintain the principles of sustainable development. It means that all activities should be within the balance of environmental, social, and economical dimensions. Gastronomic tourism can be served as one of the instruments for sustainable development in rural areas if it provides positive economic impact, cross-cultural connections, protects and preserves local heritage and traditions, and contributes to the sustainability of destination and their culture.

Involved parties in the development of gastronomic tourism in rural areas are tourists (both - local and foreign), service providers (for example, small entrepreneurs, farmers, and home-based producers) and public institutions. In public sector an important involvement is needed from national government, by providing laws, regulations, development guidelines, from tourism associations, by providing support in tourism route planning and marketing, and from local municipalities, by providing local tourism development strategies and further activities.

Specific activities that can be used for the sustainable development of rural areas in Latvia could be seen in two areas: activities for the private sector and activities for the public sector. The private sector should provide and promote local, authentic, seasonal, and good quality gastronomic products; provide an attractive presentation of food; and provide a reasonable price. Hosts themselves should be welcoming, enthusiastic, and believe that their local cuisine is special. Meanwhile, the public sector should be responsible for including gastronomic offers in promotional materials of the region; organizing campaigns for particular products, connected to a region or season, creating tourism appeal for existing food fairs and events and establishing and marketing routes. Although a number of gastronomic tourism co-operation activities have been launched, they are mostly organized within the scope of the Rural Tourism Association of Latvia and not all municipalities are involved. Local governments should develop local food tourism strategies, ease regulations for home-based and small craft businesses, and align various food tourism providers to the same goals.

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COORDINATION OF RURAL DEVELOPMENT: AN EXPLORATORY COMPARATIVE STUDY IN BOSNIA AND HERZEGOVINA, MONTENEGRO AND SERBIA

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Abstract

Coordinated Agriculture and Rural Development (ARD) is one of the preconditions for inclusive economic growth of rural areas of Bosnia, Montenegro and Serbia (BMS) and the integration of these countries into the European Union (EU). This paper analyses and compares the existing state of ARD coordination in BMS and the political and institutional framework that facilitates it. The required information came through an extensive review of the relevant literature and web-based questionnaire survey of 155 key officials of public, civil society and international organizations working for the ARD in BMS. Results reveal that coordination of ARD policy design, implementation and evaluation is still in its infancy in BMS and characterize a lack and/or weakness of coordination among the relevant institutions. Besides, the problem is identified during all phases of the policy cycle (i.e. design, implementation, monitoring and evaluation) and both at central as well as local level. Ineffective coordination is in part due to the lack of effective and institutionalized communication and information dissemination mechanisms, lack of a common understanding of '*rural*' and '*rural development*' concepts, and inadequacy of human resources. An effective coordination mechanism for ARD would reduce financial and transaction costs.

Keywords: *Rural development, Coordination, Bosnia, Montenegro, Serbia.*

Introduction

The Western Balkan (WB) region in general and Bosnia and Herzegovina, Montenegro and Serbia (BMS) in particular are now in a phase of consolidation and economic growth. The overall economic development went hand-in-hand with rising agricultural productivity (Volk, 2010). Nevertheless, these countries are still predominantly rural societies, primarily depending on agriculture and devoid of economic opportunities offered by industrial and service sectors. Despite its relative share declining since 2000, agriculture accounted for 7.0% of GDP in Bosnia and Herzegovina (BiH) in 2018 (EC, 2020a), 8.2% in Montenegro in 2018 (EC, 2020b) and 7.7% in Serbia in 2018 (EC, 2020c). Agriculture is also important for employment and poverty reduction in rural areas as it employed 18.0% of the total labor force in Bosnia in 2019 (ASBiH, 2019), around 8.0% in Montenegro in 2018 (EC, 2020b) and 15,9% in Serbia in 2018 (EC, 2020c).

Nevertheless, the fruits of the recent developments in industrial and service sectors are mostly limited to the urban areas in BMS; whereas, rural areas lag behind in terms of socio-economic development (European Integration Office-Serbia, 2011; FAO, 2014). According to the OECD's definition, BiH is among most rural countries in Europe as around 52% of the Bosnians live in rural areas (World Bank, 2020). In Montenegro, agriculture is by far the largest activity of the rural population (Bulatović, 2009; EC, 2011). In fact, Montenegro is an

overly rural country with less than 1% its land area qualifying as urban and about 33% of Montenegrin living in rural areas (World Bank, 2020). In Serbia also, about 44% of the total population live in the rural areas (World Bank, 2020) and a third of the active population depends at least partly on agriculture and allied sectors such as forestry, hunting and fishing sectors (EC, 2011a).

There can be no balanced development of the BMS region without devoting main attention to the rural areas. Thus Agriculture and Rural Development (ARD) would be the most pervasive and cross-cutting theme of the future development in BMS region. Policymakers in BMS hold a rather traditional view of rural development and see it mostly with the lens of agricultural development. As a result, most of their policy interventions pertain to production support leaving behind meagre funds for improving the quality of life in rural areas (Volk, 2010). On the other hand, experience in various other countries suggest a shift of rural development policies away from agriculture-only to a multi-sectoral approach that emphasizes on the transformation of rural society, what is often referred as *'the new rural paradigm'* (OECD, 2006; Gløersen et al., 2012; Mantino et al., 2010; RuDi, 2010a). Highly coordinated ARD becomes even more important for BMS taking into account that these countries are fully committed to join the EU and have made substantial changes to ARD policies as steps to facilitate their integration into the EU (FAO, 2014). BiH is at an early stage of preparation in the area of ARD (EC, 2020a). State-level strategic plan for rural development (RD) in BiH for the period 2018-2021 was adopted in 2018 but still remains to be effectively implemented. Montenegro remains moderately prepared in the area of ARD (EC, 2020b). In Serbia, the agricultural policy is only partly designed on a strategic basis and has been characterized by the increasing estrangement from the EU model of support (Bogdanov and Bozić, 2010).

Although at different stages of the accession process[11], the three BMS face similar challenges in transforming and modernizing their agri-food sectors which cannot be solved in absence of an effective coordination mechanism for ARD policies. Therefore, this paper aims at analyzing and comparing ARD coordination in BMS to create valuable insights on the issue and bring the most important piece of empirical knowledge. More specifically, it analyses the linkages between the actors involved in ARD policy cycle from its design and implementation to monitoring and evaluations and provides some recommendations for narrowing coordination for effective ARD policies in the three Balkan countries.

Material and methods

An extensive literature review and field research on governance and coordination on ARD were performed in BMS. A web-based pre-tested questionnaire survey of the representatives of public, civil society and international organizations involved in ARD in the three countries was carried out during the period 2011-2013. These organizations were the ministries, government institutions and executive agencies (*e.g.*, extension organizations); training and research centres and universities; users' organizations (*e.g.* farmers' union, cooperatives, associations, etc.); national and international non-governmental organizations (NGOs) and civil society organizations; donors and international cooperation agencies; financial institutions; international organizations; etc.

Key questions included cooperation and coordination with other public, civil society and international organizations having stake in ARD. Additional inquiries were about the main political, technical/bureaucratic and strategic constraints that hamper coordination between organizations dealing with RD and/or render it ineffective at each phase of the policy cycle. Conflicts between the different actors were also investigated. Respondents identified also the

[11] Montenegro and Serbia are candidate countries while Bosnia and Herzegovina is a potential candidate country for EU membership.

organization that should assume, according to them, the leadership in coordinating RD issues in each studied country. Apart from coordination among actors, the questionnaire dealt also with coordination of sectoral policies in the rural areas of BMS. Respondents were also asked about their membership in national, regional, European or international ARD networks. Considering that ARD thinking is now tilted towards inclusive approach, the questionnaire also investigated if there is perception among respondents about any organization or actor group that is actually not or insufficiently involved in the ARD policy cycle and that should be involved more actively.

Systematic "quality assurance" indicators were applied in order to minimize errors in data collection and to ensure that results can be documented as valid, reliable and comparable. These quantitative indicators (namely response rates and questionnaires with missing data proportions) were used to provide essential information about the quality of the survey (UN, 2005). Responses having missing data and those received after due date were excluded from analysis, which left 155 responses to be included in final analysis.

Quantitative data collected through the questionnaire survey were analysed using descriptive statistics (e.g. means, percentages, minima, and maxima) through Excel spreadsheets. The national ARD coordination was analysed and discussed for each studied country separately and comparison between countries was made. In tables, data were aggregated and organized in subgroups taking into consideration similarities between items. Frequencies of items mentioned by respondents were marked as following: +++ = High Importance (mentioned by >50% of respondents); ++ = Moderate Importance (25-50%); + = Low Importance (>25%). To depict a common picture of ARD coordination problems and their impact on the state of ARD in BMS, a problem tree or cause-effect diagnosis was also performed.

After the analysis of survey data, the last step was the validation and restitution phase. Summary of the results were shared with all respondents as well as with national experts on ARD for their commentary. The feedback provided by them was useful for checking the relevance and the accuracy of the results as well as the interpretation of the findings. Received feedback was particularly important to ascertain that the results of the on-line survey reflect reality in terms of ARD coordination in BMS and confirmation that no major issue has been omitted.

Results and discussion

Coordination of agriculture and rural development in Bosnia and Herzegovina

The majority of the respondents (75.7%) evaluate the coordination of sectoral policies in Bosnian rural areas as poor and/or very poor. No actor characterizes it as excellent or very good while only 9.6% consider that it is good. Just some public state and local organizations consider that ARD is well coordinated. As for sectoral policies that have good synergies with RD policy, more than a half of respondents mentioned some policies (47.5%) while some opined that policies which work together and combine their effects do not exist in Bosnia (11.5%). Except agricultural policy, tourism sectoral policies are widely recognized to have good interaction with RD policy (72.4%). Other sectoral policies mentioned by respondents include: regional development, energy, forestry, human and religious rights, gender, culture and sport, and education. Almost all the interviewees (93.4%) answered that they have had relationships with public institutions while most of them have had relationships with civil society and international organizations (86.8%). Two thirds (67.2%) of institutions contacted have collaborated with at least five public institutions, 60.7% of them have collaborated with at least five civil society organizations while 27.8% of them have collaborated with at least five international organizations. The most mentioned public institutions are Entity ministries of Agriculture, Forestry and Water Management of the Republic of Srpska (RS) and the

Federation of Bosnia and Herzegovina (FBiH)[12], the Ministry of Foreign Trade and Economic Relations (MoFTER), the Ministry of Tourism and Trade of RS, local governments, and regional and local development agencies. Among national NGOs, the most cited are the Cooperative Union of RS and FBiH and NGO "*Local Development Initiative (LIR) - Banja Luka*" while the most frequent answers regarding international development organizations and agencies referred to GTZ, USAID, Unity and COoperation for the DEvelopment of Peoples (UCODEP), SNV (Dutch Development Agency), UNDP, *Cooperazione Italiana allo sviluppo*, Regional Rural Development Standing Working Group (SWG - RRD), FAO and IFAD.

Respondents also mentioned some institutions with which they have had some conflicts. It is interesting to note that public institutions have mainly conflicts with governmental organisations and some international agencies while civil society organisations, also due to their nature, present a lower degree of involvement in conflicts.

Only 53% of the respondents identified an organization as having the leadership in coordinating RD issues while 19.7% of respondents did not recognise any leader organization; 27.8% of them did not provide any answer. The institutions more widely identified as the most important in coordinating RD issues were the Entity's Ministries for Agriculture while no public institution or civil society organization considered the MoFTER as the leader institution regarding these issues.

Around 60.7% of the respondents evaluate the coordination among the different actors as effective, around 19.7% of them evaluate it as ineffective and the rest (19.7%) did not provide any answer.

The main obstacles influencing coordination among involved actors in the design and implementation of RD policies mentioned by the respondents are included in Table 1. Overall, most of the interviewees identified the main constraints in coordination among the different organizations dealing with rural development as political (40.9%), technical (60.7%) and strategic (80.3%). Some respondent also emphasized that in some cases competition destroy cooperation thus resulting in a major constraint in coordinating RD.

Coordination of RD seems more difficult taking into consideration diverse opinions on what is meant by rural areas and RD. Most of the respondents consider RD as a cross-sectoral issue (83.6%) that includes the agricultural sector, while some of them (6.6%) provided their own definitions. No actor characterized rural development as a part of agricultural sector.

Coordination of ARD in Montenegro

About two-fifth (38.5%) of the respondents evaluated the coordination of sectoral policies in Montenegrin rural areas as good, half as poor, while some (7.7%) as very poor. Nobody considered coordination of sectoral policies as excellent or very good. Apart from agricultural policy, tourism policies are widely recognized to have good synergies with RD policy (81%), then economic (19%), environmental (19%) and water management (13%) policies. Other sectoral policies mentioned by the respondents include: energy, regional development, human and minority rights, forestry, transport, social policies, hunting economy, fiscal policies, culture and sport and education. Some also highlighted the importance of mountain and rural tourism, eco-tourism, regional branding of agricultural products, and organic production. Almost all interviewees (92%) have had relationships with public institutions while most of them have had relationships with civil society (65%) and international organizations (73%). All public institutions at the state level cooperate with international organizations engaged in RD and just 33% of local institutions. About 35% of respondents contacted have collaborated with at least 5 public institutions, 57% with at least 5 civil society organizations while 17%

[12] Bosnia and Herzegovina (BiH) consists of two governing entities i.e. the Federation of Bosnia and Herzegovina (FBiH) and Republika Srpska (RS).

collaborated with at least 5 international organizations. The most mentioned public institutions are Montenegrin Ministry of Agriculture and Rural Development, Biotechnical Faculty and local governments. Also some NGOs were cited such as the Centre for Agricultural Development (Bijelo Polje municipality), Cooperative Union of Montenegro and Agro-group (Niksic municipality) while the most cited international organizations and agencies are FAO, UNDP, COSV, World Bank (WB) and Austrian Development Agency (ADA).

Some respondents (19%) mentioned some institutions they have had conflicts with. It is interesting to note that public institutions (this refers to institutions at the state level as 100% of local institutions claimed that there are no conflicts with other organizations) have mainly conflicts with other governmental institutions mainly due to untimely submission of data. Civil society organisations present a lower degree of involvement in those conflicts. Also, some national civil society organizations stated that there are sometimes some misunderstandings with other NGOs and some public institutions.

Only 66% of respondents identified an organization as having the leadership in coordinating RD issues. The institution more widely identified as the most important in providing coordination of RD issues is the Ministry of Agriculture and Rural Development (62%) while some NGOs considered the Union of Cooperatives of Montenegro as leader institution regarding these issues.

Just 4% of respondents define the coordination among the different actors as effective while around 19% define it as ineffective and 77% as partly effective. The main constraints and problems regarding the coordination of RD policy are included in Table 1. Overall most of the interviewees identified the main constraints in coordinating the different organizations dealing with RD as political (27%), technical (46%) and strategic (46%).

It seems that there is no common understanding of what is rural area and RD and this fact makes coordination of RD policies more complicated. Most of the respondents consider RD as a cross-sectoral issue (69%) that includes the agricultural sector, while some of them (*e.g.* Cooperative Union of Montenegro) provided their own definitions. Still, quite a high percentage considers RD as a subsector of agricultural development (31%). The highest opinions diversity was among public institutions as 53% consider RD as cross-sectoral, while 47% as a subsector of agricultural development (44% of state and 50% of local institutions share the opinion that RD is part of agricultural development). All international and civil society organizations consider RD as a cross-sectoral issue.

ARD coordination in Serbia

The majority (75%) evaluate the coordination of sectoral policies in Serbian rural areas as poor and/or very poor. Nobody characterizes this issue as excellent while 16.2% consider that as good and 1.5% as very good. As for sectoral policies that have good synergies with RD policy, more than a half of respondents mentioned some policies (58.6%) while some denied existence of such policies in Serbia (14.7%). Among those that answered, apart from agricultural one, tourism policies are widely recognized to have good synergy with RD policy (52.5%) as well as regional development (22.5%), social (17.5%), economic (12.5%), environmental (12.5%) and SME's development (13%) policies. Other sectoral policies mentioned by respondents include energy (renewable sources); natural resources management; quality; culture and sport; and education.

Almost all interviewees (85.3%) had relations with public institutions. The most mentioned institutions are the Serbian Ministry of Agriculture, Forestry and Water Management as well as agricultural extension service and local authorities. More than a half of respondents (60.3%) confirmed collaboration with NGOs. The most cited civil society organizations are the national Rural Development Network and Cooperative Union of Serbia. Almost a half of

the respondents (41.2%) maintain relations with international organizations and agencies. The most cited international organizations and agencies are the European Rural Development Network, WB, FAO, USAID, GIZ, etc. More than a fourth of respondents (27.9%) also mentioned some institutions with which they have had some conflicts. It is interesting to note that public institutions (94.7%) and NGOs (100%) have mainly conflicts with governmental organisations while international organizations and agencies are less involved in those conflicts.

Almost a half of the respondents (47.1%) identified an institution as having the leading role in coordinating rural development issues. The institution often mentioned by interviewees is the state Ministry for Agriculture, Forestry and Water Management (65.6%). All surveyed international organizations and agencies considered this ministry as the leading institution in this field. Quite a few respondents from public state and local institutions considered some other organizations as the most important and recognized them as the leading actors on Serbia's RD scene - e.g. Standing Conference of Towns and Municipalities, Institute for Science Application in Agriculture, Centre for Environment and Sustainable Development (CEKOR) and Serbian Chamber of Commerce - while several national NGOs mentioned the Serbian Association of Agricultural Economists. More than a fifth (20.6%) does not recognize any institution/organization as a leading one in RD.

All the respondents consider coordination between actors involved in the RD policy cycle as only partly effective (66.2%) or merely ineffective (33.8%). The share of actors that consider coordination as ineffective is higher among civil society organizations and international development agencies with respect to public institutions. Most of national (70%) and local (65%) public institutions judge coordination as partially effective. Coordination is a problem at central as well as local levels (Table 1).

The majority of the interviewees identified the main constraints in coordination among the different organizations dealing with RD as political (67.6%), technical (64.7%) and strategic (77.9%).

The majority of respondents characterize RD as a cross-sectoral issue (70.6%) that includes the agricultural sector while 16.2% of them consider RD as a part of agricultural sector (72.7% of these are public state and local institutions). It is interesting that mainly extension service regional offices share opinion that agricultural sector includes RD. In addition, some interviewees (*e.g.* GIZ; Faculty of Agriculture, University of Novi Sad; Institute for Agricultural Economics) provided their own definitions. All surveyed international organizations and agencies consider RD as a cross-sectoral issue.

Table 1. Major causes of ineffective coordination of RD policy in BMS.

Identified Problem	**Country**		
	Bosnia	**Montenegro**	**Serbia**
Knowledge and human capital			
Lack of knowledge and information	+++	+++	+++
Lack of understanding of rural issues and priorities	+	-	++
Lack of qualified human resources	+++	+++	+++
Consultation, communication and participation process			
Lack of vertical and horizontal cooperation and communication	+++	+++	+++
Lack of cooperation willingness, interest and motivation	++	+	++
Insufficient involvement of regional development agencies		+	
Absence of a dialogue culture and participatory approaches	++	+	++
Lack of a common development platform	+	+	++

Barriers in communication and information sharing among actors	+	-	++
Contradictory approaches to RD	+	-	+
Different level of technical and administrative culture	+	+	+
Policy theory and practice gaps			
No new state Strategy for Rural Development	++	+	+
Lack of clearly defined plans, initiatives and long-term strategies	+	++	++
Unclear dynamics of the integration process and stakeholders' roles	+	-	+
Inappropriate mechanisms and procedures used	+	+	++
Lack of a common vision and autism of sectoral policies	+	+	++
Unbalanced and fragmented policy			
Mismatch between ARD policies, programs and projects	-	+	-
Weak harmonization of local, regional and national policies	++	++	-
Unclear national priorities and undefined long-term RD goals	+	-	+
Political context and administrative set up			
Institutional complexity and fragmentation	+++	+	
Existence of parallel institutions and overlapping of competences	+	+	++
Ineffective public administration at all levels	+++	+++	+++
Delay in the establishment of some relevant coordination structures	+	-	-
Urban bias reflecting political agenda setting and resulting in poor planning and budgeting for ARD	+	-	-
General politicization and political staffing	+++	++	++
Slow decentralization process and high reliance on the central level	-	+	+
Political and economic instability	++	+	+

*Source: Questionnaire survey results. +++ = High Importance (mentioned by >50% of respondents); ++ = Moderate Importance (25-50%); + = Low Importance (>25%).

Coordination of RD policy in BMS: comparative analysis

There are many commonalities related to ineffective coordination of RD policies among BMS. The analysis of the relationships and linkages between the institutions that are involved in the design and implementation of RD policy in all three studied countries showed a lack and/or weakness of coordination and that problem is faced during all the three phases of the policy cycle. A typical manifestation of ARD coordination problem in BMS and its causes and impacts is shown in Figure 1. Nevertheless, the intensity of any particular problem may vary between countries and such differences are shown in Table 1. These differences, their causes and impacts are elaborated in the succeeding paragraphs.

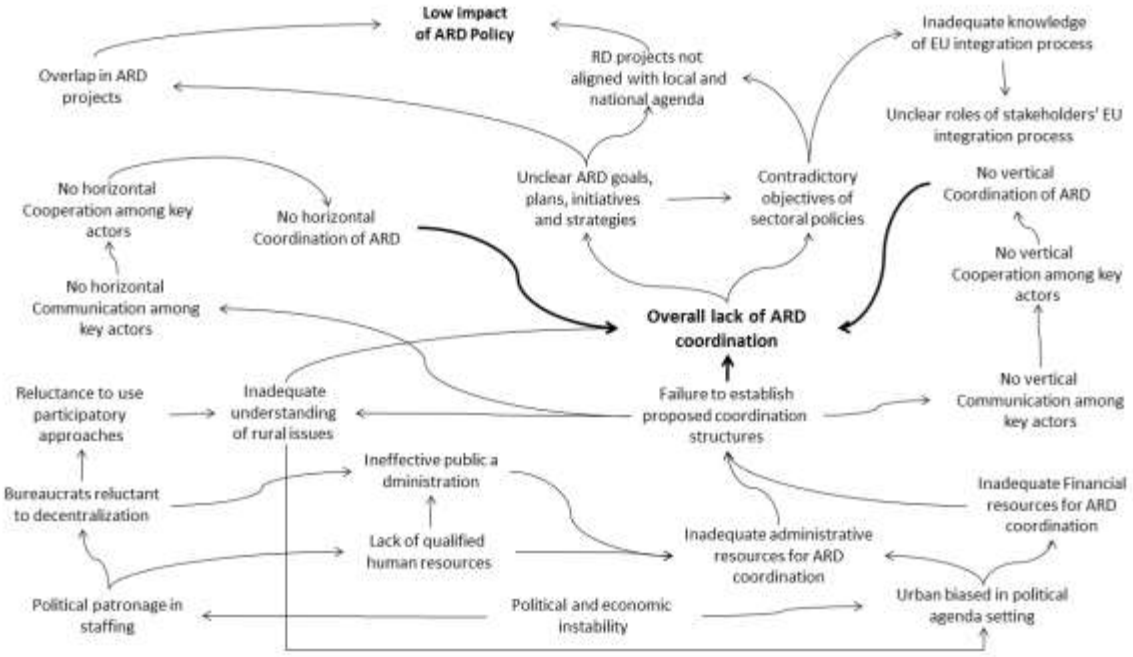


Figure 12. Root causes and impacts of poorly coordinated ARD policies.

Ineffective coordination is in part due to the lack of effective and institutionalized communication and information dissemination mechanisms. One of the actions to improve coordination would be to encourage dialogue between the involved actors by developing strong partnerships. While "formal dialogue" does exist between some public institutions and with some international NGOs and donors, it seems that a lot need to be done in order to involve civil society and private sector organizations especially during the design and formulation phase. Institutional cooperation especially between local governments and non-governmental sectors is poor (Žeravčić, 2008). That is critical especially regarding the participation of rural people, farmers and their organizations. Developing strong partnership through vertical governance arrangements and public-civil society partnering agreements can make governance institutions responsible by virtue of their participation in decision making regarding the design and implementation of RD policies (OECD, 2006).

Coordination is a problem at central as well as local level. In Serbian and Montenegrin cases, at the central level, inter-ministerial cooperation is partial rather than comprehensive because of the assumption that the most responsible institutions for RD is the Ministry of Agriculture.

Due to the lack of effective coordination there is no integrated policy implementation. Ministry of Agriculture at the national level in Montenegro and Serbia and ministries of agriculture at the entity level in Bosnia have somehow "monopoly" to design ARD policy. Even if civil society organizations rise up their voice and insist on their inclusiveness in policy design, there is still a slight resistance. Cross-sectoral cooperation is not institutionalized at the state level and it is left to individual initiatives thus depends on the good will of the staff. Incomplete and inappropriate decentralization hampers vertical coordination even between public institutions. The lack of a clear strategy for devolution limits the involvement of civil society organizations in RD issues. Public-civil society and public-private partnerships, that would make smoother coordination, are still rather experimental in most of rural areas. The lack of a public institution that can deal with coordination of all sectoral policies in rural areas is considered as a serious problem that impacts negatively rural population's livelihoods and quality of life. Of course, cooperation cannot exist without a clear legal framework, political will and orientation as well as willingness of organizations' staff to cooperate. That means that financial and human

resources should be allocated not only to implement sectoral programs but also to improve the interface and interaction between them. For that, there is a need to move towards integrated RD approaches.

Despite recurrent discourses about participation and bottom-up approaches in governance and the policy arena, the spectrum of actors involved in the different stages of the policy cycle in the three countries remain limited. In particular, the involvement of the private sector and civil society organisations in rural policy is generally still marginal. This is particularly true at central level (cf. entity level in BiH) while the situation is much better at local level.

Among others, one of the obstacles that hamper coordination between civil society organizations and between them and public institutions is a lack of a common understanding of what is "rural" and what is "rural development". The survey results suggest that despite the fact that most of the contacted organisations consider RD cross-sectoral they mean different concepts when they talk about RD. Therefore, they use different approaches and they have different priorities, which makes difficult to have a common vision for a mutual identification, formulation, implementation, monitoring and evaluation of RD projects and programmes. In order to strengthen coordination and synergy between institutions in BMS it is also necessary to further harmonize laws and regulations with the RD measures of the EU. That can make it easier also for international donors and NGOs to build their strategies for ARD in BMS.

Human capital has also a strategic relevance in order to achieve a good coordination between involved institutions. In fact, institutions' staff can operate in such a way to reduce transaction costs and to render communication smoother and flow of information faster. Communication and exchange of information could be made more effective thanks to the new ICT tools. It is critical to strengthen staff capacities, particularly the analytical and communication skills. In fact, since rural policy is strongly knowledge-based and involves multiple actors, coordination and communication mechanisms play a key role in the design and implementation of place-based RD policies. Communication should be developed horizontally as well as vertically across different government tiers (OECD, 2006). Motivation and incentives to public institutions' staff can help in achieving this objective. That would allow strengthening coordination and cooperation between them thus ensuring effective and efficient implementation of policies, programs, action plans and strategies and avoiding overlapping in responsibilities and activities, which would allow also saving precious financial resources especially in times of financial and economic crisis.

Improved coordination at the local level, by establishing forums to put together NGOs and public institutions, can positively affect and solve many existing problems in rural areas but also those faced in policy cycle phases. A better coordination between involved institutions means not only to reduce institutions operating and transaction costs but also to manage effectively incentives and subsidies provided to farmers and rural dwellers. Coordination is easier when all institutions have access to all information regarding RD that's why it is crucial to speed up the establishment of information systems. A stronger partnership between Bosnian, Serbian and Montenegrin institutions dealing with RD and those of the EU and its Member States can help to ensure a better cross-fertilization and exchange between them, which can have positive impacts on their *modus operandi*. It is not the case of a specific model transfer but the adoption of best practices and solutions that should to be remodelled considering the specific institutional, political, social and economic context of studied countries in general and their rural areas in particular.

Conclusions

Coordination between national and local level institutions involved in the design and implementation of ARD policy is still challenging in all three countries. There is a weak

institutional framework for rural policy coordination. Inadequate institutional arrangements for policy coordination had often led to a duplication of effort and general inefficiency in resource use among agencies and ministries. Improving policy coordination and consistency will contribute to addressing major territorial inequalities and foster RD. Effective, efficient and sustainable ARD policy requires a good vertical and horizontal coordination between and among multilevel governance institutions. For rural policy to be more effective, small fragmented programmes should be replaced by an integrated RD policy, that rural farm and non-farm households can benefit from, which requires a strong horizontal coordination effort at all governance levels. The coordination of sectoral policies in rural areas seems an obstacle that affects implementation. Some agricultural and rural policies and programs tend to be mutually antagonistic rather than being mutually complementary and reinforcing. Coordination of RD policy should be improved by, among others, designing and implementing mechanisms, processes and procedures aiming at upgrading communication and increasing interaction between the involved actors in the policy cycle. A basic action to strengthen coordination would be to encourage dialogue between these institutions. Communication should be developed horizontally as well as vertically across different government tiers. The new ICTs can help making communication and exchange of information more effective. Participation of civil society organizations and the private sector in RD policy design and evaluation should be encouraged. Although, participation is likely to make ARD more inclusive and comprehensive, it would create complexity in terms of setting objectives and implementing proposed measures. To tackle with this problem, ARD need high degree of coordination under the umbrella of proposed institutional arrangements. Building the capacity of human resources dealing with ARD policy is a priority. Cooperation and coordination among different sectors dealing with ARD can lead to substantial improvements but they can flourish just in case if human and institutional challenges are overcome. Decision makers in all sectors should push for more innovation and cross-sectoral participation to produce better outcomes. Integrated approach to ARD (taking into account economic, social and environmental dimensions) by strengthening inter-sectorial approaches and cooperation and coordination between local actors is highly requested.

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GOVERNANCE OF AGRICULTURAL AND RURAL DEVELOPMENT POLICY CYCLE IN BOSNIA, MONTENEGRO AND SERBIA: A COMPARATIVE ANALYSIS

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Abstract

Agriculture is important for its contribution to gross domestic product, employment and poverty reduction in rural areas of Bosnia, Montenegro and Serbia (BMS). Agricultural and rural development (ARD) processes should be well governed in order to yield expected outcomes and impacts. Governance comprises mechanisms, institutions and processes for making and implementing decisions. Governance is relevant for rural development both as process and as structure. The paper makes a comparative analysis of problems regarding the governance of ARD policy cycle (i.e. design, implementation, and monitoring and evaluation) in BMS. Research is based on an extended literature review and questionnaire surveys. Questionnaires were answered via email by 73 actors in Bosnia, 35 in Montenegro and 77 in Serbia. Respondents included representatives of public, civil society and international organizations dealing with ARD issues. Frequencies of problems mentioned by respondents were marked as following: A = High Importance (mentioned by >50% of respondents); B = Moderate Importance (25-50%); C = Low Importance (>25%). Problems regarding each stage of the ARD policy cycle were clustered. The main problem categories regard consultation, communication and participation process; fragmented, unstable and unbalanced policy; knowledge and human capital; policy theory and practice gaps; political context and administrative set up; and social and economic issues. Problems faced by the actors involved in ARD policy cycle change according to the policy cycle phase and actors' categories (public, civil society, international). Nevertheless, they are mainly related to other actors' attitude, agendas and policies and/or to procedures and the legal environment. Improved ARD policy implies optimising complementarities between public, civil society and private stakeholders. A better coordination of ARD can help solving most of the identified governance problems. There is a strong need for mobilization of all rural stakeholders and economic actors through appropriate governance arrangements.

Keywords: *Governance, Rural development, Policy cycle, Bosnia, Montenegro, Serbia.*

Introduction

Bosnia and Herzegovina, Montenegro and Serbia (BMS) share at least two commonalities, which have strong implications for their future development. First, these countries are predominantly rural and in many respects agriculture is still important for these economies. The share of agriculture and fisheries in total GDP accounted for 7% in Bosnia and Herzegovina (BiH) in 2018 (EC, 2020a), 8.2% in Montenegro in 2018 (EC, 2020b) and 7.7% in Serbia in 2018 (EC, 2020c). The sector also employed 17.9% of the total labour force in Bosnia in 2019 (ASBiH, 2019), around 8% in Montenegro in 2018 (EU, 2020b) and 15.9% in Serbia in 2018 (EC, 2020c). Besides, around 52% of the Bosnians, 33% of the Montenegrins and 44% of the Serbians live in rural areas (World Bank, 2020). The second important

commonality among BMS is their desire for integration in the European Union (EU) despite the fact they vary in terms of their progress towards this objective. Montenegro and Serbia are officially candidate countries, and BiH is just a potential candidate. The EU integration experience of other countries show that agricultural and rural development (ARD) is one of the most demanding subject with a highly extensive set of obligations, as it alone accounts for approximately 40% of the total EU body of Law (*Acquis communautaire*) (FAO, 2014; Lampietti et al., 2009). The third important similarity among BMS is somewhat shared cultural, institutional and social values as these countries had been ex-republics of the former Yugoslavia. These similarities make BMS highly suitable for comparative analysis of their governance of ARD policy cycle (i.e. design, implementation, and monitoring and evaluation) and is the objective of this paper. Such analysis is highly important not only because it will result in generating insights on the governance of ARD policy in each country but also because it will inform the policymakers in these countries about their relative position and give them a chance to learn from each other.

Material and Methods

An electronic administered questionnaire was used for analysing problems related to ARD policy cycle governance. Online questionnaire was pretested in all studied countries with representatives of public, civil society and private sector. The selection of eligible actors was mainly based on internet search, literature review and personal connections and communications. In order to follow on-line questionnaire delivery process, message delivery notification system and message read receipt option were used (Table 1).

Table 1. Delivery proportion of sent emails

	Total number of sent e-mails	Emails successfully delivered*		Emails successfully delivered and read by the receiver	
		No.	%	No	%**
Bosnia	283	174	61.5	120	69.0
Montenegro	117	78	66.7	50	64.1
Serbia	314	162	51.6	120	74.1

Source: Authors' elaboration

* % of emails successfully delivered in total number of sent e-mails

** % of emails successfully delivered and read by the receiver in total number of successfully delivered emails

Questionnaires were carried out with representatives of public, civil society and international organizations dealing with ARD in the three target countries, e.g. ministries, government institutions and executive agencies (*e.g.* extension organizations, etc.); public structures (training and research centres, universities, etc.); users' organizations (*e.g.* farmers' union, cooperatives, associations, etc.); national and international NGOs and civil society organizations (CSOs); donors and international cooperation agencies; financial institutions; international organizations, etc. Key open-ended questions included the operational level and the geographical coverage of each organization, involvement of the organization in a RD policy and/or project and in which phase of the policy cycle, the main problems in their rural areas as well as regarding RD policy design, implementation and evaluation. After survey results analysis, the last step in the survey research process was the validation and restitution phase. Relevant information from the survey were gathered, analysed and shared with all respondents as well as national and international experts on ARD. Feedback provided by them was useful for checking the relevance and the accuracy of the results. Quantitative data collected through the questionnaire survey were analysed using descriptive statistics (e.g. means, percentages, minima, maxima) through Excel spreadsheets.

Results and discussion

Problems in rural areas in general and those related to governance in particular influence not only the impact of policies but also the different phases of policy cycle. Problems should be known in order to design effective policies. One of the common problems faced in rural areas of the three target countries is that related to low human capital. This has implications in terms of policy design, implementation as well as monitoring and evaluation. The main issue is a lack of a stable agricultural and rural policy. ARD policy-making in the WB region in general and in BMS in particular has often been dictated by ad-hoc considerations. Regardless of the differences among the three countries, the pragmatic ad-hoc approach for defining measures prevails. Agricultural policy is still implemented mostly based on annual programs of budget allocation, which are not stable in terms of funds, support measures and eligibility criteria. Rural development (RD) policy is generally subordinate to production support (Volk, 2010). One characteristic specific to BiH is the complexity of its state administration, which complicates the implementation of its agricultural policy. Agricultural policies greatly differ, both by level of funds and structure of measures. Therefore, their implementation is far away from the model in EU. Legal harmonization is at the initial stage, and the institutional capacities of a modern state are still being built (Bajramović *et al.*, 2010). A stronger harmonization with the objectives and instruments of the CAP is required to strengthen the European integration process in Montenegro (MARD, 2012). Montenegro has already undergone significant changes in the process of reforming agricultural policy at the level of creating a legal framework and formulating strategic guidelines. However, two main challenges remain: to build up the implementation structure; and to provide appropriate budgetary allocation to support the agricultural sector (Marković and Marković, 2010). In Serbia, the implementation of agricultural policy has been permanently changing. Programs and regulations were changed several times during the year. The agricultural policy in Serbia is only partly designed on a strategic basis and has been characterized by the increasing estrangement from the EU model of support. Frequent changes in administrative structures brought radical changes in the support system (Bogdanov and Bozić, 2010). Actors face different problems during the different phases of the RD policy cycle in BMS. Problems are also different depending on the responding actor typology (*i.e.* public, civil society or international organizations) as well as their geographical coverage and level of operation (*i.e.* local, national or international). However, it was noticed that sometimes it is not clear for many institutions the boundary of the different rural policy phases.

Policy formulation and design

The main common problems that actors in BMS face during RD policy design (Table 2) include lack of funds and expertise as well as weak participation of rural people and farmers; due also to a slow regionalization and decentralization processes especially in Montenegro and Serbia. A higher level of private and civil society sectors participation in RD policy design is required and it should be additionally encouraged. There is a deficit regarding human capital in the competent institutions especially public ones. Local governments should be more opened to democratize the design process and include all local stakeholders (FAO-ROECA, 2012). Governance decentralization and rural service delivery are important for sustainable ARD. Decentralized political system would better answer local communities' needs. Strengthening and improving service providers' capacities will directly affect the supply and quality of services (IFPRI, 2007). A vibrant service sector is vital for a prosperous local economy and crucial for meeting the rural population's needs (OECD, 2010b).

Lack of integrated policy planning and incompatibility of sectoral policies in rural areas is also a constraint that is aggravated by the absence of municipal rural development strategies

and low capacity at local level. Some institutions have different understanding of RD concept and what is meant by rural areas as there are many, even public institutions, that consider agricultural and RD as the same. This is influenced by mind-set of officers due, among others, to the legacy to the previous Yugoslav system. Overall, different understandings of rural development influence policy design as it results in strategies, mainly at the local and regional levels, that are designed with a high orientation to agriculture development in all three studied countries. The problem of funding limitation supposes also a prioritization of development targets which, very often, is not done in a proper way. For rural policy to be more effective, small fragmented programmes should be replaced by an integrated RD policy, that rural farm and non-farm households can benefit from, which requires a strong horizontal coordination effort at all governance levels. Support programmes, subsidies and easier access to credit are necessary to support diversification activities and fully utilise the potential of rural areas.
National inter-organizational cooperation and inter-donors collaboration among those involved in policy design as well as communication with end-users based on bottom-up approach, in order to get information about their real needs at the local level, remain weak, which leads to non-effective policy planning and overlapping of activities. Incoherent and non-harmonized policy planning among state, regional and local levels hinders desirable development of rural areas in BMS. Lack of reliable and accurate data on ARD make even more complex and demanding policy design process itself. Political and economic instability in BMS indicates that more attention should be given to pragmatic planning made by competent people for the well-being of the future generations, without any political influence. That's true especially for Bosnia where is obvious a lack of political consensus at the state level due to decentralized political system in which real power is at the entity level as well as due to the legacy of the civil war and different interests among stakeholders because of cultural and religious differences. Policy design should be in line with political, economic, environmental, social and cultural environment of the different rural areas in each country. Policy environment should be analysed at the earliest stages of the design of any new policy. Analysis of existing policies must cover national, regional (entity level in BiH) or local levels. It is clear that specific approach to rural areas development, that will consider geographical specificities of rural territories in BMS, could be the only successful solution that will work in the reality. To enhance ownership and sustainability it is crucial to emphasize the responsibility of the local and regional actors in policy planning and implementation. For ensuring the participation of end-users and local actors in policy design, participatory planning or collaborative planning approaches should be adopted. In case of a planning phase where only technical expertise is used, it must be complemented by collaborative or participatory methods in the following stages to ensure rural population's active participation. Despite recurrent discourses about participation and bottom-up approaches in governance and the policy arena, the spectrum of involved actors remains limited. In particular, the involvement of the private sector and civil society organisations in rural policy design and formulation is generally still marginal. This is particularly true at central level (entity level in BiH) while the situation is much better at local level. Moreover, the funds at disposal of local councils and administrations are very low and this limits the scope of their interventions.

Policy implementation and service delivery

The largest part of the surveyed organizations are involved in policy implementation, especially public local institutions and CSOs. The main problems faced during RD policy implementation (Table 3) include shortage of financial resources and weak human capital. Indeed, the capacity of local public institutions remain low and there are many institutional constraints to apply the participatory approach (FAO-ROECA, 2012).

Table 2. Problems in ARD policy design in Bosnia, Montenegro and Serbia: Commonalities and differences

No.	Identified Problem	Country		
		Bosnia	**Montenegro**	**Serbia**
	Knowledge and human capital			
1.	Lack of highly qualified human resources and poor administrative capacities	A	A	A
2.	Lack of knowledge and information about needs at the local level	B		B
3.	Lack of local and regional RD strategies and low level of local actors' capacity	A	B	B
4.	Unfamiliarity of local actors with local resources			C
5.	Lack of accurate and reliable databases for detailed problem analysis in rural areas	A	A	A
6.	Low capacity of administration and incompetence of policy design makers	B	C	C
7.	Lack of appropriate sectoral analyses and unrealistic assessment of all resources			C
	Consultation, communication and participation			
8.	Insufficient involvement of all stakeholders in policy design, especially target groups	A	A	A
9.	Difficulties to find common interest among stakeholders	C		C
10.	Disinterest, lack of initiatives and reluctance of rural population and local governments	C		B
11.	Lack of horizontal and vertical coordination and communication	A	A	A
	Policy, theory and practice gaps			
12.	Copying of policies (instruments and measures) without checking their effects		B	C
13.	Mismatching between policy development and its content at the local and national levels	B	B	B
14.	Lack of clear rural development goals and strategic planning			C
15.	Lack of a clear vision for rural areas development	C		
16.	No clear definition of rural areas and misunderstanding of RD concept	C	C	C
	Fragmented and unbalanced policy design			
17.	Excessive focus on the agricultural sector and tourism	A	A	B
18.	Lack of integrated policy planning and incompatibility of sectoral policies	C	C	B
19.	No consideration for rural territorial specificities (e.g. specific approach)	C	C	C
20.	No distinction made between rural development and agriculture	A	A	A
	Political context and administrative set up			
21.	Political instability and great influence on policy design made by political parties	A	B	A
22.	Centralized political system and slow process of decentralization		C	C
23.	Institutional complexity, fragmentation and overlapping of responsibilities	B	C	C
24.	Lack of funds for policy design	A	B	A
25.	Lack of coordination between the government and donors and huge bureaucracy	C	C	

*Source: Authors' elaboration based on the questionnaire survey results.

Legend: *A = High Importance* (mentioned by >50% of respondents); *B = Moderate Importance* (25-50%); *C = Low Importance* (>25%).

Weaknesses regarding policy design have also consequences for policy implementation. For instance, the lack of a clear long-term vision leads to policy discontinuity, which affects negatively rural population's interest and, consequently, its involvement and cooperation. Inappropriate measures that do not meet the needs of the rural population are related in a large extent to policy design. What often happens is that policy design is done by experts without involvement of end-users or hearing their opinions. During the policy design and implementation should be considered municipal specific characteristics due to different levels of socio-economic development and rural economy diversification.

The main problem pointed out by local actors is the lack of funding and/or complicated application procedures. Lack of funds at the local level is due to limited budget that was further decreased also due to economic crisis. Although insufficient, available funds at local level are not always efficiently used.

Lack of responsibility related to activities failure makes implementation approach uncontrolled. Public officers and employers should be at all times accountable to the end-users. Therefore, it is important to improve the ability of the rural population to demand services and hold service providers accountable (IFPRI, 2007b).

The coordination of sectoral policies in rural areas seems an obstacle that affects implementation. Not only sectoral policies are not implemented in a coordinated way in rural areas but also there are weak synergies with donor initiatives. Moreover, bureaucratic problems render things even more complicated for rural people and implementation of RD initiatives more difficult. In order to improve implementation, it is important to have clear and well-designed policies but also receptive and well prepared rural population as well as a good staff dealing with the implementation process that is able and willing to solve punctual problems and difficulties. Regionalization should allow solving many of the problems regarding RD policy implementation by allowing strengthening the sub-national governance levels capacities.

Lack of technical resources and human resources with various backgrounds is an obvious problem in BMS. For successful implementation is crucial to have competent personnel with various backgrounds such as sociology, communication, project management, RD, rural economy diversification, background related to legal issues, etc. Insufficiently developed capacities at the local level are due to problems regarding education in rural areas. Rural areas very often experience a lack of schools or they are of lower quality, resulting in lower educational achievements of rural population that affect the development of human assets (FAO, 2010). Responsibility is not only on government acts but also on non-governmental organizations that did not do their part of the job, despite funding from international donors.

Available human capacities that deal with ARD within municipalities mainly consist of just one person that is not capable to mentor rural population due to time and large territory constraints. Lack of spatial plans at the local level is a problem recorded in all three studied countries that influence land tenure security (land ownership) and further investments on the ground. Coordination at the local level by establishing forums to put together NGOs and public institutions can positively affect and solve many existing problems. Due to lack of coordination, there is lack of integrated policy implementation. Ministry of Agriculture at the national level in Montenegro and Serbia and ministries of agriculture at the entity level in Bosnia have "monopoly" to design ARD policy. Even CSOs rise up their voice and insist on their inclusiveness in policy design, there is still a slight resistance.

Policy monitoring and evaluation

Public institutions in BMS are much more involved in monitoring and evaluation activities in comparison with civil society sector and international organizations. One of the main problems is related to the human and technical capacities of institutions dealing with

monitoring and evaluation tasks in all studied countries (Table 4). In addition, there is also the problem of the lack of quantifiable targets. Methods and approaches adopted in project monitoring are also considered inappropriate. The lack of clearly defined and shared procedures of and indicators for RD policy monitoring and evaluation (M&E) is a patent constraint to improving the design and implementation of ARD policies in BMS. M&E activities cannot be performed on a proper way if non-standardized, inappropriate, too complicated and time consuming indicators are used. Inefficient monitoring has negative impact on evaluation of impact of policies. In fact, that means lack of appropriate use of ICT, low quality of collected data and that there is not a sufficient feedback on policies especially from beneficiaries and end-users as well as local actors.

As far as policy M&E is concerned, the advantages of control can be offset by a loss of trust; lower-level actors may prefer to have an impeccable formal record rather than to be effective and innovative. Therefore, the benefits of evaluation must be balanced against their administrative demands on governments and farmers. The system should be sophisticated yet also manageable (Zahrnt, 2010). Political parties that are in power can influence evaluation process, jeopardize its independence and affect validity of obtained results as they decide from whom information will be taken. However, some evaluation problems are clear-cut and remedies are within reach. For example, the data needs of evaluators could be better integrated into routine statistics and evaluators could be made more independent from policymakers. Unstable and sensitive political situation affects M&E process. In BMS, there is the culture that M&E is done only after completing policy implementation so there are often no funds for this activity or there is no good will due to change of governments and their priorities. Sometimes objectives that are set for policy are not easy to grasp and quantify using simple set of indicators. Policy objectives should be spelled out clearly and guidance on suitable indicators should be provided directly in the legislation establishing the policy instrument (Zahrnt, 2010). Currently, objectives are often left vague. Furthermore, the number of policy objectives and instruments should be limited as high number of indicators to assess rural development favours box-ticking over thorough analysis.

Conclusions

Problems faced by the actors involved in the RD policy cycle change according to the policy cycle phase and actors' categories (public, civil society, international) as well as their operation level (national, local). Nevertheless, they are mainly related to other actors' attitude, agendas and/or to procedures and the legal and institutional environment. Rural policy effectiveness in the three countries is undermined by many governance problems and constraints that decrease the policy impacts. One of the major constraints to policy effectiveness is policy instability. Instability of policy governance in the three countries affects negatively its real impact in rural areas. Another problem is related to the inconsistency in policies. Another issue is the weak institutional framework for rural policy coordination. A further factor that may help explaining low impact of rural policies is the poor implementation. Poor managerial capacity, bureaucratic bottlenecks, corruption and high rates of policy turnover tend to aggravate the problem of policy implementation. Effective, efficient and sustainable ARD policy requires a good vertical and horizontal coordination between and among multilevel governance institutions. Participation of CSOs and the private sector in RD policy design and evaluation should be encouraged. Building the capacity of human resources dealing with ARD policy is a priority. Governance requires leadership over all levels - from national and regional to local. Adopting an integrated approach to ARD and strengthening intersectorial approaches as well as cooperation and coordination between local actors are highly requested.

Table 3. Problems in ARD policy implementation in BMS: Commonalities and differences

No.	Identified Problem	Country		
		Bosnia	**Montenegro**	**Serbia**
	Knowledge and human capital			
1.	Lack of information flow and insufficient education and preparedness of the rural population	A	A	A
2.	Insufficiently developed capacities at the local level	C		B
3.	Lack of technical resources and human resources with various backgrounds	A	A	A
4.	Low administrative capacities at all levels	C	B	B
	Consultation, communication and participation			
6.	Lack of horizontal and vertical coordination and collaboration	A	A	A
7.	Low accessibility to end-users and their lack of interest	C		C
9.	Mentality and prejudices of the local population and lack of initial capital for end-users	C		**B**
10.	Dominance of personal and short-term interests over common and long-term interests	C	C	C
11.	Bank sector is not involved enough		C	
	Policy theory and practice gaps			
12.	Inappropriate measures not meeting needs of the rural population	C		B
	Social and economic issues			
14.	Lack of initial capital for end-users	C		B
15.	Reluctance of locals regarding modern technologies use	B		C
16.	Lack of support to rural entrepreneurship		C	
	Fragmented, unstable and unbalanced policy			
17.	Absence of a regional approach and lack of clearly defined plans	C	C	
18.	Poor project adjustments to specific situations in rural areas			C
19.	Lack of integrated policy implementation	B	B	A
20.	No continuity in the policy implementation	C	C	B
23.	Policy incoherence and inconsistency	C	C	B
	Political context and administrative set up			
24.	Political instability, disorganization, corruption, rivalry among institutions and mutual mistrust	B	B	B
25.	High political influence on decisions and absence of political will	B	B	A
26.	High level of administrative and bureaucratic requirements	B	A	B
27.	Donor initiatives are not always consistent and overall financial support is not well targeted	C	C	
28.	Technical and bureaucratic barriers and high administrative costs	B	B	B
29.	Overlapping of activities and responsibilities	C	C	B
30.	Lack of good credit policy and incentive measures	B	C	
33.	Lack of local institutional support	C		B
34.	Limited budget and complicated application procedures for grants awarding	B	C	A

*Source: Authors' elaboration based on the questionnaire survey results.

Legend: **A** = **High Importance** (mentioned by >50% of respondents); **B** = **Moderate Importance** (25-50%); **C** = **Low Importance** (>25%).

Table 4. Problems in ARD policy monitoring and evaluation in BMS: Commonalities and differences

No.	Identified Problem	Country		
		Bosnia	**Montenegro**	**Serbia**
	Knowledge and human capital			
1.	Low education level of end-users	A	B	A
2.	Lack of knowledge and experience	B	B	B
3.	Lack of well-trained staff and personnel for M&E and competent structures	B	B	A
	Consultation, communication and participation process			
4.	Lack of interest of the local population	B		B
5.	Poor vertical and horizontal coordination and cooperation	A	A	A
6.	Lack of transparent information on performed M&E activities	C	C	C
7.	Lack of ownership over M&E process by rural communities			C
8.	Underdeveloped information system for data collection and analysis	C	C	
	Policy theory and practice gaps			
9.	Lack of standardized indicators, methods and simple procedures for M&E	C	B	A
10.	Unreliable feedback from beneficiaries	B	B	A
11.	Mixing of RD measures with direct payments			C
12.	Lack of an appropriate framework and orientation on short-term goals			C
13.	Unrecognized importance of M&E activities	C		A
14.	Difficult evaluation of unquantified and immeasurable objectives			C
15.	Difficult measurability of some indicators and quantifying results of individual measures			C
16.	Lack of interest of local authorities	C		B
	Political context and administrative set up			
17.	Political instability	A	B	A
18.	Influence of political parties on M&E process jeopardizing its independence	B	C	B
19.	Favouritism among institutions and superficiality	C		C
20.	Lack of control structures	C	C	B
21.	Lack of continuity in monitoring	C	C	
22.	Conflicts of interest	C	C	
23.	Undeveloped programs implementation control		C	C
24.	Corruption and bribery	C	C	
25.	Lack of financial resources for M&E activities	B	C	B

*Source: Authors' elaboration based on the questionnaire survey results.

Legend: *A = High Importance* (mentioned by >50% of respondents); *B = Moderate Importance* (25-50%); *C = Low Importance* (>25%)

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FORESTRY AND AGRO-FORESTRY

CHARACTERISTICS OF HUMOFLUVISOL SOIL IN THE MIDDLE DANUBE REGION AND ITS PRODUCTION CAPACITY FOR POPLAR CULTIVATION

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Abstract

The paper presents the physical and chemical properties of humofluvisol soil. The survey was made on a geographical stretch from Novi Sad to Titel, in the area of the embankment protected part of the alluvial plain of the Danube. The granulometric composition of the pedological profiles examined shows the highest fraction of fine sand fraction, which averages from 44.39 to 59.98%. The average content of total sand from 45.28 to 61.64%, that is, the content of total clay from 38.36 to 54.72%. The textural classes of the surveyed soils are in humus A horizon up to a depth of 45 cm: loam and silty clay loam, and in the lower parts of the profile depths from 40 cm to 210 cm: clay loam, loam, sandy loam, loamy sand and sand. The pH values increase with depth, ranging from 7.96 to 8.31, which classifies them in moderately alkaline soils. The humus content is highest in the surface humus A horizon from 1.94 to 4.60%, that is, it is weak in this horizon to very humus soils. The average humus values are in the range of 1.71 to 2.43%, so they are weak to moderately humus soils. According to the granulometric composition, the tested soil has favorable water-air properties, with wetting exclusively through groundwater. The production potential of this soil is optimal for the plantation production of a different range of black poplar clones.

Keywords: *Humofluvisol, Granulometric composition, Hydromorphic soil, Danube*

Introduction

On the alluvial plane of the Danube river are represented hydromorphic soils, from which in tearms of productivity is significant the class of semigley soils, the soil type humofluvisol, For semigley soils, different synonyms occur as alluvial semi rendzinas according to Antić et al. (1967), or meadow black soils, according to Nejgebauer (1952). According to the accepted classification of Škorić et al. (1985), a synonym for humofluvisol is alluvial meadow soil. In the area of inundations, Miljković (1996) distinguished three parts: coastal, central and pre terrace. The central part of the alluvial plain is characterized by widespread semigley soil with meadow and forest vegetation, and according to Antić et al. (1968) here occurs a mixed forest of poplar, elm, black alder and common dogwood, and the *Alnetum-qlutinosae, Alneto-salicetum, Ulmeto-quercetum, Populetum-albae Fraxinetum* communities. After the construction of the protective embankment along the middle course of the Danube, the soil in the protected part of the alluvial plane is exposed to wetting only by groundwater, which in this area oscillates from 1 to 2 m. According to Pekeč et al. (2012a) mean ground water depth on humofluvisol soil ranges from 234 to 260 cm, with a variation amplitude of 138 to 265 cm. Due to its favorable water-air properties and moistening by ground water, humofluvisol soils have great fertility potential. Flood protection has created hydrological conditions that enable intensive plantation production of various clones of black poplar. The production potential of this habitat, with the use of new varieties and modern cultivation technology, will provide significant quantities of wood for the needs of industry, energy and local needs of the population in the Republic of Serbia with the possibility of significant quantities for export

according to Marković (2000). The existing poplar wood processing industry has significant capacities, and there are opportunities for profitable exports, especially to the neighboring countries of Western Europe where this raw material is in short supply (Marković 2000). This paper aims to show the physical and chemical properties of this soil in the Middle Danube region and its production capacity for intensive poplar cultivation.

Material and method

In this paper was investigated soil in the protected area of the Danube alluvial plain. Three pedological profiles were opened on the route from Novi Sad to Titel. External and internal morphology of profile was described. Soil samples were taken from opened pedological profiles and the following physical and chemical soil analyzes were performed:
Mechanical composition, B-pipette method with preparation of samples for analysis in sodium pyrophosphate according to Thun, and the textural soil class was determined according to the Tommerup classification;
$CaCO_3$ content was determined volumetrically using a Scheibler calcimeter;
pH was determined in a suspension of soil with water, potentiometric;
The content of humus by Tyurin, modified by Simakov;
Total nitrogen according Kjeldahl;
Easily accessible phosphorus and potassium according to the AL method, Egner-Riehm - Dominigo.
Based on the analyzes performed, the characteristics of the tested soil are presented



Figure 1 Location of pedological profiles

Results and discussion

According to the presented granulometric composition of the examined pedological profiles (Table 1), the largest fraction of fine sand was in range from 44.39 to 59.98%. Silt fraction values are represented in a smaller proportion, which ranges from 27.25 to 39.35%. The content of clay fraction was much less represented in values from 11.11 to 15.37%, while the content of coarse sand was the lowest, i.e. in the range from 0.89 to 7.51%. It can be stated that the average content of total sand is from 45.28 to 61.64%, and the content of total clay is from 38.36 to 54.72%.

Table 1 Granulometric composition

Profil	Horizon	Depth (cm)	Coarse sand (%)	Fine Sand (%)	Silt (%)	Clay (%)	Total sand (%)	Total clay (%)	Tekstural class
P1 Kać	Amo	0-40	2.53	37.55	47.2	12.72	40.08	59.92	Loam
	C	40-100	0.21	31.75	46.72	21.32	31.96	68.04	Loam
	Gso	100-200	0.88	77.68	14.32	7.12	78.56	21.44	Loamy sand
	Gr	> 200	3.01	92.95	0.76	3.28	95.96	4.04	Sand
	Average		1.66	59.98	27.25	11.11	61.64	38.36	
P2 Titel	Aa	0-42	0.74	31.06	48.84	19.36	31.80	68.20	Silt clay loam
	Cca	42-100	0.66	60.94	27.44	10.96	61.60	38.40	Loam
	Gso	100-155	1.27	41.17	41.76	15.80	42.44	57.56	Clay loam
	Average		0.89	44.39	39.35	15.37	45.28	54.72	
P3 Novi Sad	A	0-45	2.16	49.80	28.28	19.76	51.96	48.04	Loam
	C	45-85	0.15	57.17	27.48	15.20	57.32	42.68	Sandy loam
	Gso	85-170	0.01	36.91	41.80	21.28	36.92	63.08	Loam
	Gr	170-210	27.73	61.31	7.12	3.84	89.04	10.96	Sand
	Average		7.51	51.30	26.17	15.02	58.81	41.19	

The texture classes of the surveyed soils are in the humus A horizon: loam and silty clay loam, in the C horizon: loam and sandy loam, while Gso are sub horizons of glay: loamy sand, loam and clay loam, and Gr sub horizons are textural class: sand.

Table 2 Chemical composition

Profil	Horizon	Depth (cm)	$CaCO_3$ (%)	pH (H_2O)	Humus (%)	Total N (%)	P_2O_5 (mg/100g)	K_2O (mg/100g)
P1 Kać	Amo	0-40	18.05	7.61	3.68	0.292	19.4	63.5
	C,sa	40-100	17.23	7.75	2.06	0.051	3.5	10.5
	Gso	100-200	14.77	8.24	0.71	0.083	2.9	4.5
	Gr	> 200	11.90	8.23	0.78	0.023	3.1	4.0
	Average		15.48	7.96	1.81	0.115	7.2	20.6
P2 Titel	Aa	0-42	5.63	7.93	4,60	0.295	5.2	18.2
	Cca	42-100	27.01	8.30	1.15	0.099	0.8	9.5
	Gso	100-155	28.8	8.57	1.55	0.133	0.2	10.0
	Average		20.31	8.27	2.43	0.176	2.7	12.5
P3 Novi Sad	A	0-45	7.82	7.93	1.94	0.076	10.5	8.5
	C	45-85	8.23	8.53	1.66	0.001	9.5	7.7
	Gso	85-170	4.53	8.51	2.81	0.003	13.2	10.7
	Gr	170-210	4.94	8.27	0.43	0.000	3.9	2.9
	Average		6.38	8.31	1.71	0.020	9.3	7.4

Analyzing the chemical properties of the investigated humofluvisol soils can be stated the average value of carbonates in the range from 6.38 to 20.31%, showing that these soils are carbonate to strongly carbonate ones (classification Belić et al. 2014). pH values increased with depth and ranged from 7.96 to 8.31 which classifies these soils as moderately alkaline soils according to the American classification. The humus content is highest in the surface humus A horizon with 1.94 - 4.60% (weak, moderate to very humous) and decreases with depth of profile, while the average humus values are in the range from 1.71 to 2.43% that is, weak to moderately humous soil (Scheffer-Schachtschabel classification). The content of total nitrogen follows the content of humus in the soil where the highest N values are in the humus horizons, and with soil depth its value decreases. The average values of total nitrogen are from 0.020 to 0.176%, that is, very poor to well supplied by nitrogen according to the Wohltmann classes. In the tested soils, the readily available phosphorus content is in range from 2.7 to 9.3 mg/100g, and the readily available potassium content ranged from 7.4 to 20.6 mg/100g, and it can be stated that these soils are poorly provided with readily available

phosphorus and weak to well supplied with readily available potassium, according to the Egner-Riehm classification. Analyzing the surveyed soils it can be concluded that the depth of the humus horizon is up to 45 cm.

The physiological depth of these soils is up to 170 cm and 200 cm respectively, which is very favorable for the development of forest tree roots. Below this depth is the Gr sub horizon of gley, which is always directly influenced by groundwater moistening. Groundwater oscillates with the depth of the profile as indicated by the Gso sub horizon and reaches the highest level, which is the upper boundary of this sub horizon, up to 85 or 100 cm from the surface. Given the favorable silt and clay content, both C horizons and surface A horizons are moistened during this period by capillary climbing of water due to high groundwater. It can be stated that the physiologically active part of the profile is supplied directly by groundwater or moistened by capillary climbing to higher horizons. The average content of total clay of the surveyed soils is in the range of 38.36 to 54.72%, which indicates favorable water-air properties, i.e. favorable ratio of non-capillary and capillary pores, capillary climbing of water and content of accessible water for plants. According to the tested properties of this soil, it has high potential for growing of black poplar. Research by Živanov (1977) shows that the most important property of soil for the cultivation of poplar is the content of the silt + clay fraction in the physiologically active part of the profile. The same author states that the best productivity can be achieved if the participation of this fraction is 30-50%, and the data of the examined soils are within the stated limits with minor deviations. Also, all soil fertility indicators depend on the part of this fraction.

According to Pekeč et al. (2012b) in the physiologically active part of the pedological profile humofluvisol soil has a favorable ratio of total sand to total clay, and therefore favorable physical properties. Herpka et al. (1990) stated that in the group of the best soils for poplar cultivation (humofluvisol on fossil soil), the average annual volume increment for clone I-214 was up to 32.8 m^3/ha. During the study about the production capabilities of Eurasian poplar clones in relation to soil properties, Rončević et al. (1999), came to the conclusion that relatively high wood volume per hectare is related to the silt + clay content as well as to the amount of easily accessible water. The favorable granulometric composition of the tested soils influences the optimal water-air properties of these soils. Considering the influence of groundwater and moistening the profile of these soils directly with groundwater or moistening the upper layers through capillary climbing, it can be concluded that the tested soils are optimally provided with moisture. Based on the facts presented, it can be concluded that these are favorable soils with optimal production capacity for establishing plantations of various clones of black poplar.

Conclusion

The paper presents the properties of humofluvisol soil at sites from Novi Sad to Titel, located in a protected part of the alluvial plane. According to the granulometric composition, these soils are characterized by the largest fraction of fine sand. The content of total sand ranges from 45.28 to 61.64%, and the content of total clay from 38.36 to 54.72%. The textural classes of the surveyed soils in the physiologically active layer are loam, clay loam, silty clay loam, sandy loam and loamy sand, while the lowest part of the texture class profile is sand. According to the chemical composition, the studied soils were moderately alkaline, weak to moderately humous, in the classes from very poor to well supplied with nitrogen, poorly provided with readily available phosphorus, and poorly to well provided with readily available potassium. The granulometric composition of the tested soils affects their optimal water-air properties, and the moistening of these soils is in the lower sub-horizons directly by groundwater or indirectly by moistening the upper horizons by capillary climbing. The

physical and chemical properties of the investigated humofluvisol soils, and the supplying of these soils with moisture through groundwater, indicate that these are the optimal soils for establishing plantations of various clones of black poplar.

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THE ABILITY OF VEGETATIVE REPRODUCTION OF HORNBEAM IN THE PROCESS OF NATURAL REGENERATION OF THE SESSILE OAK FORESTS IN NORTHEASTERN SERBIA

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Abstract

Hornbeam is one of the most represented undergrowth species in the sessile oak stands in the area of northeastern Serbia. In this paper, a comparative analysis of the growth characteristics of three-year-old and four-year-old sessile oak seedlings and three-year-old and four-year-old hornbeam shoots and root suckers in the sessile oak stand in the area of Majdanpek in northeastern Serbia was performed. From the aspect of phytocenological classification, the stand is defined as an association of sessile oak with hairy sedge (*Carici pilosae – Quercetum petraeae* B. Jov. 1989) on eutric brown soil on neutral and basic eruptive rocks. The combination preparatory and seed cut was conducted in 2013 when the undergrowth of accompanying tree species (white linden, hornbeam, common ash) was removed, together with individual dead sessile oak trees. Subsequently, a large number of shoots and root suckers of species from the undergrowth (mostly white linden and hornbeam) appeared and affected the growth and development of the sessile oak seedlings. The average number of hornbeam shoots per one stump was 6.5 and of root suckers 4.0. Compared to the average height of three-year-old sessile oak seedlings, the average height of three-year-old shoots was 125.6 cm higher, as for root suckers, 131.2 cm higher. Compared to the average height of four-year-old sessile oak seedlings, the average height of four-year-old shoots was 174.7 cm higher, as for root suckers, 198.9 cm higher. Differences in heights indicate a significant lag in the growth of sessile oak seedlings in comparison to the shoots and root suckers of hornbeam, which can significantly affect the outcome of the regeneration of these stands.

Keywords: *Northeastern Serbia, Vegetative propagation, Hornbeam, Sessile oak, Natural regeneration.*

Introduction

Sessile oak forests have a very important place in the forestry of Serbia and Europe considering their ecological significance, distribution, productivity, as well as the quality of the products obtained by their use.

In Serbia, sessile oak forests are located at altitudes 300-1300 m, while in northeastern Serbia, sessile oak forests are located at altitudes 300-700 (800) m, where they alternate with submontane beech forest that occurs at colder exposures.

As a species that does not tolerate shading, sessile oak is able to develop exclusively in the first (dominant) floor which creates space for the development of a large number of undergrowth species, and later is an aggravating factor in the regeneration of these forests. This is also indicated by the composition of these forests where the sessile oak as the main species is represented by 72.5%, while the accompanying species (Turkey oak, hornbeam,

beech, manna ash, common ash, silver linden, Hungarian oak, and field maple) are represented by 27.5% (Banković *et al.*, 2009).

In the area of northeastern Serbia, the share of mature and overmature stands in the total area of high sessile oak forests is significant (59%), and based on that, the natural regeneration of these forests is a significant silvicultural problem. Mostly these are overmature stands, which are over 160 years old and with incomplete canopy which create the conditions for the occurrence of undergrowth species with pronounced biological strength and clear perspective to occupy habitats of sessile oak (Kanjevac, 2020).

Hornbeam (*Carpinus betulus* L.) is one of the most represented undergrowth species in the sessile oak stands. Considering that hornbeam is species which can tolerate shade, as well as that after removing the trees of the parent stand has a very pronounced ability to regenerate vegetatively, in the process of regeneration of sessile oak stands hornbeam is a very important competitive species which has the ability to occupy these habitats (Krstić *et al.*, 2005; Röhrig *et al.*, 2006; Petrović, 2010; Krstić and Petrović, 2011; Březina and Dobrovolný, 2011; Matula *et al.*, 2012; Sikkema *et al.*, 2016; Kanjevac, 2020). The ability of vegetative reproduction of hornbeam was previously studied by Babić (2006), Babić and Krstić (2007), Krstić and Petrović (2011), Matula *et al.* (2012), Kanjevac (2020), etc.

In the process of natural regeneration of the sessile oak forests with hornbeam, large number of shoots and root suckers appear after cutting hornbeam trees from the undergrowth, which endanger the survival and further development of sessile oak seedlings (Krstić and Petrović, 2011; Kanjevac, 2020). The ability of vegetative reproduction of hornbeam increase from younger to older trees, i.e., from smaller to larger stumps, while for the oldest trees it is manifested in almost 100% of cases (Matula *et al.*, 2012). Therefore, sessile oak in the stands with hornbeam cannot be regenerated if the silvicultural treatment is concentrated only on the trees of the parent stand, but it is necessary to remove shoots and root suckers of hornbeam which have the potential to overgrow and overcome sessile oak seedlings (von Lüpke, 1998; Ligot *et al.*, 2013; Kanjevac, 2020).

Based on the above, the aim of this paper is to analyze the ability of vegetative reproduction of hornbeam to point out the importance of this species as a competitor in the process of natural regeneration of sessile oak forests.

Material and Methods

The research was performed in the sessile oak stand in the area of Majdanpek in northeastern Serbia, in an experimental field 0.25 ha (50x50 m) in size.

The studied stand is located in the management unit "Ujevac" at an altitude between 320 and 350 m, on slope up to 25º, and western exposure. From the aspect of phytocenological classification, the stand is defined as an association of sessile oak with hairy sedge (*Carici pilosae – Quercetum petraeae* B. Jov. 1989). The soil is deep eutric brown, formed on neutral and basic eruptive rocks, weakly skeletal.

The combination preparatory and seed cut was conducted in 2013 when the undergrowth of accompanying tree species (white linden, hornbeam, common ash) was removed, together with individual dead sessile oak trees. The canopy of the stand after this cut is incomplete (0.5-0.6). Subsequently, a large number of shoots and root suckers of species from the undergrowth (mostly white linden and hornbeam) appeared and affected the growth and development of the sessile oak seedlings (Figure 1).

At the end of vegetation periods 2016 and 2017, on 15 stumps of hornbeam of the same generation and different dimensions, at the age of 3 and 4 years, a study of the ability of vegetative reproduction of this species was performed.

The dimensions of the stumps (diameter and height of the stump) were measured, and the number and height of hornbeam shoots and root suckers were determined. The height of hornbeam shoots and root suckers was determined at different ages (3 and 4 years).
For the purpose of comparative analysis of the growth of sessile oak seedlings and shoots and root suckers of hornbeam, the number and growth characteristics of sessile oak seedlings (root collar diameter and height) were studied on 30 measurement squares (basic units) of 1 m^2 in size, within the experimental field. Considering that the majority of sessile oak seedlings originated from the mast year of 2012, at the end of the vegetation periods 2015 and 2016 the number and growth characteristics of three-year-old and four-year-old seedlings were studied.



Figure 1 The studied stand 4 years after the preparatory-seed cut (photo: Kanjevac, 2017)

Results and discussion

The average number of 3-year-old seedlings per square meter was 7.7, the maximum was 32 and the minimum 0. The average number of 4-year-old seedlings per square meter was 7.4, the maximum was 32 and the minimum 0. The average height of 3-year-old seedlings was 25.2 cm. Their maximum height was 45.0 cm and minimum 5.0 cm. For 4-year-old seedlings, the average height was 30.8 cm, the maximum was 65.0 cm and the minimum 8.0 cm. The average diameter of 3-year-old seedlings was 3.4 mm, with the maximum 6.0 mm and the minimum 1.0 mm. For 4-year-old seedlings, the average diameter was 3.9 mm, the maximum was 8.0 mm and the minimum 1.0 mm (Table 1).

Table 1 The number and growth characteristics of sessile oak seedlings at different ages

Element	Statistical parameter	Age	
		3	**4**
Number of seedlings (per m^2)	min	0	0
	max	32	32
	$\bar{x}$	7.7	7.4
	std. error	1.58	1.43
	cv (%)	112.61	105.56
Height (cm)	min	5.0	8.0
	max	45.0	65.0
	$\bar{x}$	25.2	30.8
	std. error	0.79	1.13
	cv (%)	34.53	45.34
Diameter (mm)	min	1.0	1.0
	max	6.0	8.0
	$\bar{x}$	3.4	3.9
	std. error	0.09	0.14
	cv (%)	30.14	44.67

Table 2 Stump dimensions and the number of shoots and root suckers of hornbeam

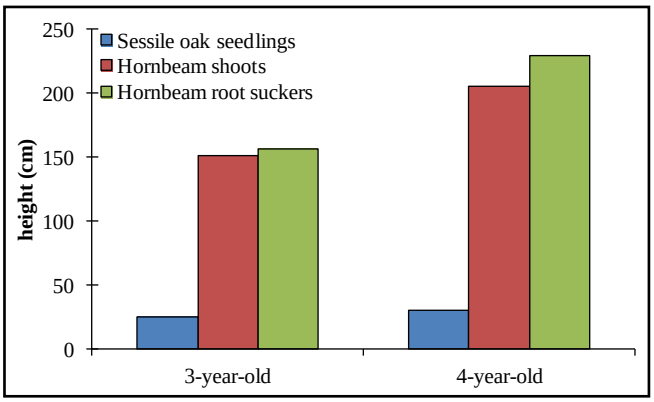
Stat. paramet.	Stump diameter d (cm)	Stump height h (cm)	Number of shoots	Number of root suckers	Number of shoots and root suckers
min	6.0	6.0	1	1	4
max	20.5	23.0	15	9	23
mean	12.2	13.4	6.5	4.0	10.5
std. error	0.98	0.92	0.91	0.42	1.22
cv (%)	35.9	30.7	62.6	47.3	51.8

The diameter of the analyzed stumps ranged from 6.0 cm to 20.5 cm, and the mean diameter was 12.2 cm. The height of the analyzed stumps ranged from 6.0 cm to 23.0 cm, and the mean height was 13.4 cm. The minimum number of shoots per one stump was 1, the maximum was 15, while the average number was 6.5. The minimum number of root suckers per one stump was 1, the maximum was 9, and the average number was 4.0. Altogether, the minimum number of shoots and root suckers per one stump was 4, the maximum was 23, while the average number was 10.5 (Table 2). In the research of the ability of vegetative reproduction of hornbeam in the habitat of hygrophilous forests of common oak, hornbeam and narrow-leafed ash in the area of Srem in Serbia, it was found that under other equal conditions, in high hornbeam forests, the ability of vegetative regeneration is equally manifested regardless of tree felling time (Babić, 2006). The minimum height of three-year-old shoots of hornbeam was 75.0 cm, the maximum was 216.0 cm, while the average height was 150.8 cm. For four-year-old shoots, the minimum height was 81.0 cm, the maximum was 303.0 cm, and the average height was 205.5 cm. When it comes to three-year-old root suckers of hornbeam, the minimum height was 80.0 cm, the maximum was 222.0 cm, and the average height was 156.4 cm. For four-year-old root suckers, the minimum height was 130.0 cm, the maximum was 338.0 cm, while the average height was 229.7 cm (Table 3). In the same sessile oak stand, it was found that the shoots and root suckers of white linden (*Tilia tomentosa* L.) have very intense growth in the initial phase of development, whereby the heights of three-year-old shoots and root suckers of white linden ranged from 77-370 cm (the average height was 242.3 cm), and the heights of four-year-old shoots and root suckers ranged from 90-430 cm (the average height was 290.8 cm) (Kanjevac and Babić, 2017). In the area of Srem in Serbia, very

pronounced energy of growth of hornbeam shoots and root suckers was found. The maximum height of one-year-old shoots was 212 cm, and the average height was 85 cm, while the maximum height of root suckers was 164 cm, and the average height was 83 cm (Babić, 2006).

Table 3 The heights of three-year-old and four-year-old shoots and root suckers of hornbeam

Element	Statistical parameter	Age	
		3	4
Height of shoots (cm)	min	75.0	81.0
	max	216.0	303.0
	mean	150.8	205.5
	std. error	7.34	6.68
	cv (%)	23.8	30.0
Height of root suckers (cm)	min	80.0	130.0
	max	222.0	338.0
	mean	156.4	229.7
	std. error	9.25	12.95
	cv (%)	25.8	24.6



Graph 1 Heights of sessile oak seedlings and shoots and root suckers of hornbeam at different ages

Compared to the average height of three-year-old sessile oak seedlings, the average height of three-year-old shoots was 125.6 cm higher, as for root suckers, 131.2 cm higher. Compared to the average height of four-year-old sessile oak seedlings, the average height of four-year-old shoots was 174.7 cm higher, as for root suckers, 198.9 cm higher. Differences in heights indicate a significant lag in the growth of sessile oak seedlings in comparison to the shoots and root suckers of hornbeam, which can significantly affect the outcome of the regeneration of these stands (Graph 1).

Conclusions

Hornbeam is one of the most represented undergrowth species in the sessile oak stands in the area of northeastern Serbia. The obtained results indicate a very pronounced ability of vegetative reproduction of hornbeam, as well as a very intensive growth of shoots and root suckers in the initial phase of development. A comparative analysis of the growth characteristics of three-year-old and four-year-old sessile oak seedlings and three-year-old and four-year-old hornbeam shoots and root suckers indicated a significant lag in the growth of sessile oak seedlings in comparison to the shoots and root suckers of hornbeam, which can significantly affect the outcome of the regeneration of these stands

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LEVEL AND QUALITY OF GROUNDWATER IN EUGLEY SOIL OF THE CENTRAL DANUBE BASIN

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Abstract

The paper examines hydromorphic soils of the gley soil type class, eugley soil type. The tested soils are located in the protected part of the mid-Danube River basin. According to the chemical properties of the tested soils, the pH value averages from 7.60 to 8.23 and increases with depth. The total water-soluble salts of the tested soils had average values of 0.13 to 0.19%. The granulometric composition shows an increased average content of total clay, while the texture classes are loam and clay loam. The influence of the Danube water level on the groundwater level is reflected in the high correlation coefficients, which range from 0.72 to 0.92. The measured groundwater level ranged from a maximum value of 30 cm deep to +20 cm above the ground surface. The minimum groundwater depth measured was from 37 to 110 cm. The variation of groundwater during the year ranged from 55 to 80 cm. Groundwater quality classes according to U.S. Salinity Laboratories move in the spring from C3S1 to C3S3, in the summer from C4S2 to C4S3 and in the fall from C3S1 to C4S4. Groundwater quality has been declining between spring and fall. Poor groundwater quality can affect the salinisation and alkalization of the surveyed soils.

Keywords: *Eugley, Groundwater level, Groundwater quality, Danube.*

Introduction

The soils of the hydromorphic order of the middle Danube region have a different geographical position compared to the Danube riverbed. Clay soils occupy the lower parts in the area of inundation, of which the eugley soil (Gleysols according to WRB, 2006) is in the lowest groundwater areas or in depressed zones. These soils are characterized by their heavy mechanical composition, and according to Pekeč et al. (2011a) the textural classes of eugley soils in this area are loamy sand, sandy clay loam, loam and clay loam with porosity from 43.22 to 64.31%. The basic feature of these soils is groundwater, which is relatively close to the surface of the soil with a depth of approximately 1 m (Pekeč et al., 2011b). In the area of the alluvial plain flood protected by the embankment, the only source of moisture for these eugley soils comes from groundwater, while the water level of the Danube has a great influence on the movement of groundwater levels. Groundwater presents major hydromorphism factor in these soils and its level significantly their productivity. In addition to the level of groundwater and its variation throughout the year, attention should also be paid to the quality of groundwater (Nešić et al., 2003) since it can influence the properties of eugley soil. Groundwater quality as well as its level varies over different periods of the year according to Dragović et al. (2007). Hadžić, (1989), investigated the sleep regime in some hydromorphic soils under irrigation conditions in the area of northern Banat. The quality of groundwater was not satisfactory and there was an increase in soil salinity in the layer of 0-60 cm, which indicates soil damage and the need to regulate the level and quality of groundwater. The groundwater level during the test period measured in piezometers during

the vegetation period oscillated within limits that are above the critical level, which significantly affected the sonic regime in the soil. The critical level of groundwater and the danger of secondary salinization were pointed out by Negebauer (1949), as well as by Miljković, et al., (1977). Škorić, (1994), studied the groundwater regime in terms of its quality in the area of Bačka and Banat and found poor groundwater quality. Therefore, this study aims to present eugley soil properties, groundwater properties during different monitoring periods, as well as the groundwater level and the amplitude of its variation.

Material and methods

The eugley soils in the protected part of the inundation of the Mid-Danube river basin were examined. Three pedological profiles were opened on 2006 year, and their external and internal morphology described. A hydromorphic soil was defined from the soil of the gley class, the eugley soil type according to the classification of Škorić et al. (1985). Soil samples were taken for laboratory analysis and the following analyzes were performed:
Determination of soil mechanical composition by international B-pipette method with sodium pyrophosphate preparation, according to Bošnjak et al. (1997) and the determination of texture classes according to Tommerup.
Humus content in soil by the Tjurin method after Simakov's modification, (1957).
Soil chemical reaction, pH in H2O electrometrically with a glass electrode.
The content of total water-soluble salts by the method of measuring electrical conductivity in saturated earth paste.
Groundwater levels were measured using piezometers, and variations in groundwater levels were obtained by calculation.
Groundwater samples were taken in spring, summer and autumn on 2006 year, and the laboratory determined:
Dry residue by evaporation of water to 1050 C.
Electrical conductivity (EC 106 at 250 C) using a conductometer.
Sodium adsorption ratio (SAR) - calculated.
Groundwater quality classes according to U.S. Salinity laboratory (Richards, 1954) and according to the FAO classification (Ayers and Westcot, 1985).

Results and discussion

According to the chemical reaction of the tested eugley soils (Table 1), average pH values ranged from 7.60 to 8.23. An depth dependant increase in alkalinity in the lower sub-horizons of the eugley subjecting it to the class of weakly to moderately alkaline soils, according to the American Classification of chemical reaction. The humus content of these soils is expressed in surface horizons with values from 2.34 to 6.75%, classifying them as moderate to very humus (according to the Scheffer-Schachtschabel classification). Also, the entire depth of the profile revealed a certain percentage of salts, averaging from 0.13 to 0.19%. The granulometric composition of the surveyed soils shows (Table 1) that these soils have an increased average content of total clay, ranging from 61.30 to 70.26% and total sand content from 29.74 to 38.70%. The high content of total clay affects the higher proportion of fine and medium pores and the poorer vertical water permeability of eugley soil (Pekeč et al. 2013). According to the Classification of Soils of Yugoslavia (Škorić et al. 1985), poorly saline soils contain from 0.25 to 0.50% of salt, indicating that these lands are below the limit of defining poorly saline soils, but certainly with a certain percentage of salt.

Table 1 Chemical properties and granulometric composition of investigated soils

Profile	Horizon	Depth (cm)	pH (in H_2O)	Humus (%)	Total salt (%)	Total sand (%)	Total clay (%)	Texture class
1/06	Aa	0-25	7.66	2.34	0.19	35.52	64.48	Loam
	Gso	25-80	8.77	0.83	0.18	41.88	58.12	Loam
	Average		8.22	1.59	0.19	38.70	61.30	
2/06	Aa	0-35	7.60	6.75	0.15	37.80	62.2	Loam
	Average		7.60	6.75	0.15	37.80	62.20	
3/06	Aa	0-45	7.98	5.59	0.11	22.32	77.68	Clay loam
	Gr	>45	8.47	5.02	0.15	37.16	62.84	Clay loam
	Average		8.23	5.31	0.13	29.74	70.26	

Analyzing the texture classes of the surveyed soils, it can be concluded that these are heavy mechanical composition soils withwith loam prevailing in the first two profiles and clay loam in the third profile.

Table 2 Correlation of Danube levels and groundwater levels at investigated sites

Profile	Distance from Danube bed (m)	r coefficient	Relative groundwater level (cm)			
			maximum	average	minimum	amplitude
1/06	3716.00	0.92	30	80	110	80
2/06	2071.00	0.72	(+)20	18	37	57
3/06	4161.00	0.78	5	38	60	55

The distance of pedological profiles from the Danube bed (Table 2) ranged from 2071.00 to 4161.00 meters. The influence of the Danube water level on the groundwater level is evident in the significantly high correlation coefficients ranging from 0.72 to 0.92. During 2006, the Danube was extremely high (the highest since 1965 when floods were recorded), which also affected groundwater dynamics. The measured groundwater level (Table 2) ranged at a maximum value of 30 cm depth to +20 cm above the soil surface. The minimum measured groundwater depth was 37 to 110 cm and the mean value was 18 to 80 cm. Groundwater variation during the year ranged from 55 to 80 cm.

Table 3 Quality and classes of groundwater

Profile	Groundwater sampling depth (cm)	SAR	Conductivity EC (dS/m)	Dry residue (mg/l)	Water class (US Salinity Laboratory)	Need for water restriction (FAO classification)
Spring						
1/06	50	10.25	1.96	1386	C3S2	moderate
2/06	10	2.26	1.57	1191	C3S1	moderate
3/06	5	16.34	1.28	1008	C3S3	moderate
Summer						
1/06	67	8.33	11.91	2729	C4S3	possible
2/06	20	2.28	7.52	2060	C4S2	possible
3/06	38	7.69	5.46	1293	C4S3	possible
Autumn						
1/06	110	10.78	3.89	2945	C4S3	possible
2/06	16	2.17	1.69	89	C3S1	moderate
3/06	60	20.49	4.12	2189	C4S4	possible
MAQ		0-15	0-3	0-2000		

MAQ – maximum allowable quantity, SAR – Sodium apsorption ratio

Groundwater sampling was performed in three periods: spring, summer and autumn. According to the presented data (Table 3), it can be concluded that the SAR values (Sodium adsorption ratio) in the spring were from 2.26 to 16.34, in the summer from 2.28 to 8.33 and in the autumn from 2.17 to 20.49. Nešić et al. (2003) emphasize the value of SAR as an indicator of the relative activity of water-soluble natrium in adsorption reactions with soil. The electrical conductivity was in the spring from 1.28 to 1.96 dS/m, in the summer from 5.46 to 11.91 dS/m and in the autumn from 1.69 to 4.12 dS/m. Looking at all periods, the dry residue of groundwater samples ranged from 89 to 2945 mg/l. It can be seen that the conductivity and dry residue values exceeded the maximum allowable amounts in summer and autumn and the SAR values in autumn, which resulted in worse water classes during this period. Groundwater classes show by U.S. Salinity Laboratory (Richards, 1954) ranges from C3S1 to C3S3 in the spring, that is, groundwater tested is classified as saline (C3) and low in sodium (S1) to high sodium (S3), in the summer the classes moved from C4S2 to C4S3 or very saline (C4) with medium sodium content (S2) to high sodium content (S3). In the fall, the groundwater class ranged from C3S1 to C4S4 classified as salty (C3) to very salty (C4) and with low sodium content (S1) to very high sodium content (S4). According to the FAO groundwater classification, in the spring there was a moderate need for restriction of this water, in the summer there was a possible need for restriction, and in the fall a moderate and possible need for restriction (Ayers and Westcot, 1986). Groundwater levels were highest in the spring of the measurement year and decreased during the summer and fall. It can be concluded that, according to the depth of groundwater for each profile (Table2.), the water class deteriorated from spring through summer to autumn.

Values of the pH reaction of eugley soils indicate increase in the lower suborizons leading to alkaline classification Also, the entire depth of the profile revealed a certain percentage of salts, averaging from 0.13 to 0.19%, which can be associated with poor groundwater quality, which deteriorated during the monitoring period from spring to autumn. The above average high groundwater level durinh the year of monitoring has a significant impact on the salinity and alkalization of higher horizons of the tested soil. According to Dragović et al (2007), the critical groundwater level is extremely important in structural soils, and from the aspect of soil salinization, the critical groundwater level is 200 to 250 cm according to Nejgebauer (1952), while according to Miljković et al. (1977) the critical level is 225 cm for loam. Bearing in mind that the granulometric composition of these soils according to the textural classes of loam to clay loam, this gives the possibility of greater capillary climbing of water even at lower groundwater levels. Longenecker and Lyerly (1974) cite according to Dragović et al. (2007), that the capillary rise in loamy soil is from 150 to 300 cm, and in loam with very fine capillaries from 6 to 9 meters for a long period of time. Based on the lower quality of the water that is salted and alkalized, it can be concluded that groundwater can affect the salinization and alkalization of the tested soils, which some chemical properties of soil such as increased salt content and increased pH values in the lower sub-horizons and confirm.

Conclusion

The paper examines the characteristics of eugley soils, as well as the level and quality of groundwater of these soils in the Mid-Danube region. According to the chemical properties, the reaction of these soils is weak to medium alkaline and belongs to the class of weak to very humus soils, with an increased percentage of total salts. The granulometric composition of the surveyed soils shows an increased average content of total clay with textural classes of loam and clay loam. The influence of the Danube water level on the groundwater level is reflected in the high correlation coefficients. Groundwater levels ranged from a maximum of 30 cm

deep to +20 cm above the ground. The minimum value of groundwater ranged from 37 to 110 cm, and the mean value from 18 to 80 cm in depth. Groundwater variation during the year ranged from 55 to 80 cm. Groundwater quality classes according to U.S. Salinity Laboratories range from C3S1 to C3S3 in the spring, C4S2 to C4S3 in the summer and range from C3S1 to C4S4 in the fall. From spring to autumn, water quality has been declining. High groundwater levels in the year of monitoring, above the critical level, may have an effect on the salinisation and alkalization of the tested soils.

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RISK MANAGEMENT IN ACCORDANCE WITH THE PRINCIPLES OF INTERNATIONAL CERTIFICATION AND STANDARDIZATION IN FORESTRY

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Abstract

Risk management in forestry is a generic process of identification, analysis, evaluation, and decision making.Decision management and risk management in forestry are conditioned by the need to analyze the general environment (economic, ecological, social, technological and political-legislative).The process of standardization and international certification for wood intensifies in Serbia. Management of complex systems such as forestry is demanding to supervisory assessment and the establishment of risk management processes. By implementing the process of risk management according to ISO31000, forestry can have multiple benefits in the area of achieving business goals, better compliance with relevant legislation and international standards, improving management, planning, and more efficient use of resources.The process of risk management uses interactive matrices and analyzes 140 parameters.This paper aims to present the methodology of harmonization of legislation at the operational level with management functions in forestry through the process of risk management to make forest management environmentally friendly, socially acceptable, and economically sustainable.

Keywords: *forestry, risk management, standard, certification*

Introduction

Risk management in forestry is a generic process of identification, analysis, evaluation, and making of corresponding decisions.

There are many interactive influences in forestry that affect the functions of management and decision-making. Regarding the origin and purpose of forests, there were formed interactive decision-making matrices for the observed forest functions (Šijaković et al., 2013).

Using the Analytic Hierarchy Process (Saaty, 1980) and multiple iteration methods, have ranked listed forest functions with a score from one to ten by its relation to the origin and purpose of the forest. Forests, classified by purpose and origin, are grouped into ten basic categories.

Decision management and risk management in forestry are conditioned by the need to analyze the general environment (economic, ecological, social, technological and political-legislative).

The Law on Inspection Control of the Republic of Serbia defines the concept, purpose, types and forms of inspection and the concept of risk. The Law on Inspection Control of the Republic of Serbia is subject-oriented. The Forestry Law of the Republic of Serbia is object-oriented, where one subject often manages multiple objects. The process of standardization and international certification for wood intensifies in Serbia. The goal of the certification is to make the forest management environmentally friendly, socially acceptable and economically sustainable. Management of complex systems such as forestry is demanding to supervisory assessment and the establishment of risk management processes. Regarding this, the ISO31000 standard provides generic guidance for risk management.

According to ISO31000, risk management includes the process of interactive collaboration, communication and consultation, establishing criteria for process monitoring, identification,

risk analysis and risk assessment. The implementation of the risk management process is highly beneficial in the area of achieving business goals, better compliance with relevant legislation and international standards, improving management, planning, and more efficient use of resources.

What is specific for the activity of forestry is its own connection to the certain space (object). Ten basic forest functions, for the forests exposed to threats, i.e., possible risks, have been formulated. Forest functions interactively link to the spatial division according to the origin and purpose of the forest (Šarčević, 2012). Ten basic threats that can cause adverse consequences in the business and the conduct of forestry entities have been identified.

This paper aims to present the methodology of harmonization of legislation at the operational level with management functions in forestry. Through the process of risk management and introduction of wood certification, forest management can be environmentally friendly, socially acceptable, and economically sustainable.

Material and Methods

In this study I have analyzed the law regulations of the Republic of Serbia related to the process of risk management in forestry. By comparing wood certification principles and ISO31000 risk management standards, have identified basic threats (risks) to forest management.

There are many interactive influences in forestry that affect the functions of management and decision-making. Regarding the origin and purpose of forests, there were formed interactive decision-making matrices for the observed forest functions (Šijaković et al., 2013).

Table 1 shows the parameters of the multi-criteria optimization of basic forest functions (Šarčević, 2012).

Table 1. Parameters of the multi-criteria optimization of basic forest functions

Table of Basic Forest Functions		Optimalization Parameters	%
1	Impact on water regime	0,231	23,12%
2	Protection of soil from erosion, torrents and landslides	0,164	16,41%
3	General environmental protection and impact on climate through the production of oxygen and biomass	0,136	13,55%
4	Recreational functions (recreation, sport, tourism)	0,125	12,49%
5	Function of biodiversity and gene pool protection	0,111	11,11%
6	Impact on agrocomplex	0,063	6,28%
7	Health functions, air purification, noise protection	0,062	6,22%
8	Impact on hunting and wildlife	0,051	5,08%
9	Social-cultural function (protection, landscape, aesthetic, spiritual)	0,040	4,04%
10	Cultural-educational (scientific-educational) functions	0,017	1,70%

Using the Analytic Hierarchy Process (Saaty, 1980) and multiple iteration methods, have ranked listed forest functions with a score from one to ten by its relation to the origin and purpose of the forest. Forests, classified by purpose and origin, are grouped into ten basic categories. The F-valorisation coefficient of forest function regarding the origin and purpose of the forest calculates using the scalar estimation method (Šarčević, 2011).

Table 2 shows the F-valorisation coefficients of forest function by origin and forest purpose. The ISO31000 norm defines risk as it's the effect of uncertainty on the set goals (deviation

from expected - positive and/or negative). Risk is related to potential events or consequences, or their combination. Following the ISO31000 principles, there are ten basic identified threats - risks concerning forests.

Table 2: F-valorisation coefficients of forest function regarding forest origin and purpose

The forest purpose	The forest origin								
	High natural stand of hard and / or soft deciduous trees	High natural stand of conifers and deciduous trees	High natural conifer stand	Coppice natural stand of hard and / or soft hardwoods	Mixed by origin - seed and vegetative origin	Artificially raised stand of hard and / or soft deciduous trees	Protective belt and line forest	Artificially raised coniferous stand	Wallet and litter
Special nature reserve and / or biosphere reserve	9,50	8,80	8,67	8,49	8,34	8,14	7,72	7,67	5,70
Nature park, national park, regional park	9,33	8,63	8,50	8,32	8,17	7,97	7,55	7,50	5,53
Forests with priority protection function	9,07	8,37	8,24	8,06	7,91	7,71	7,29	7,24	5,27
Teaching base	8,94	8,24	8,11	7,93	7,78	7,58	7,16	7,11	5,14
Landscape of exceptional features and a monument of nature	8,80	8,10	7,98	7,79	7,64	7,45	7,03	6,98	5,01
Forest park	8,61	7,91	7,79	7,60	7,45	7,26	6,84	6,79	4,82
Game park, game reserve	8,60	7,90	7,77	7,59	7,44	7,24	6,82	6,77	4,80
Forests for recreation	8,46	7,76	7,63	7,45	7,30	7,10	6,68	6,63	4,66
Forests and forest habitats with production-protective function	8,31	7,61	7,48	7,30	7,15	6,95	6,53	6,48	4,51
Former agricultural land overgrown with forest species	7,48	6,78	6,66	6,47	6,32	6,13	5,71	5,66	3,69

Also, using the scalar method of assessment, the Risk; R-coefficients to the basics of the threats were calculated (Table 3).

Table 3: R-coefficients to the underlying threats

Basic threats - adverse effects	Risk; R- coefficients				
	1	2	3	4	5
Entomological damage	0,192	0,336	0,721	0,865	0,961
Phytopathological phenomena - diseases	0,182	0,319	0,683	0,819	0,91
Illegal logging	0,127	0,222	0,476	0,571	0,635
Management risk	0,102	0,179	0,383	0,459	0,51
Snowbreaks and windbreaks	0,099	0,173	0,371	0,445	0,494
The impact of fire	0,082	0,143	0,307	0,369	0,41
The impact of water erosion	0,068	0,120	0,257	0,308	0,342
The impact of floods	0,057	0,100	0,215	0,258	0,287
The impact of agrocomplex	0,048	0,085	0,181	0,217	0,242
The impact of hunting	0,042	0,074	0,158	0,19	0,211

The R-coefficient of risk (threat) is multiplied by the corresponding F-coefficient of the function of forests, defining the severity of possible consequences (Ci). In this way, by relating the functions of forests with the purpose and origin of forests, the risk interacts with potential threats and adverse effects on the forest.

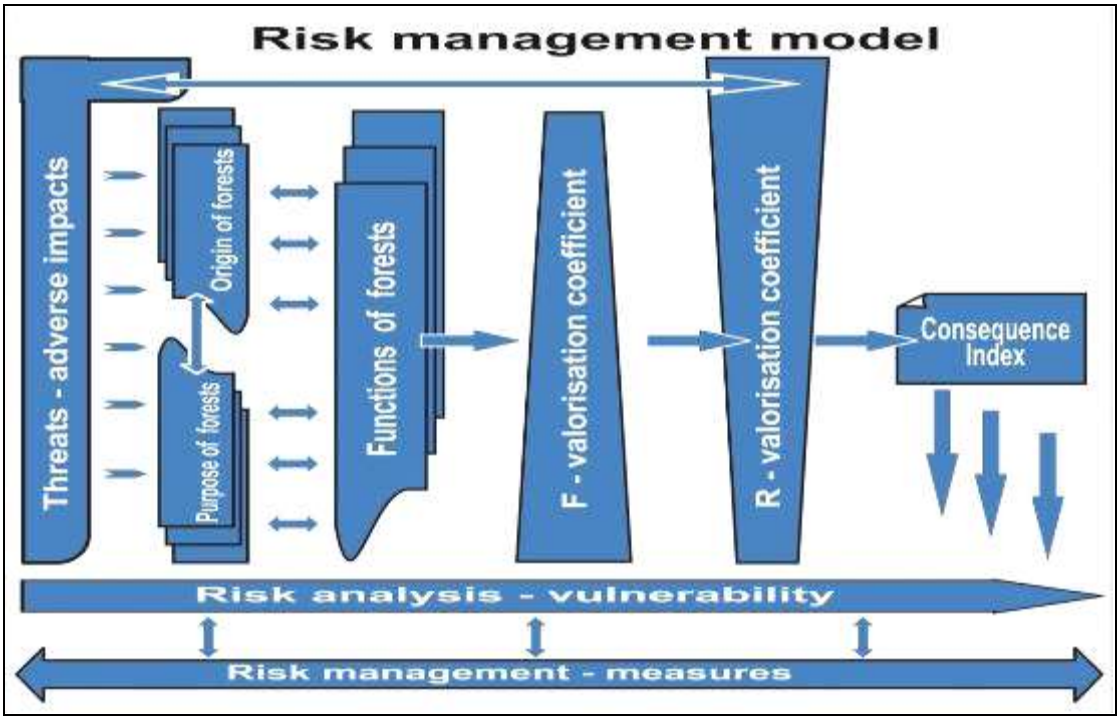
Table 4 shows the consequences index (Ci). By the method of iteration of the R and F coefficients, have defined the relationship between the range of the consequence assessment index and the expected risk.

Table 4: Consequence index (Ci)

Risk Rating	**Total consequence index ∑ (Ci)= F-coefficient x R-coefficient**	
Insignificant	to 8,600	
Low	8,601	17,000
Medium	17,001	35,610
High	**35,611**	**42,730**
Critical	42,731	47,520

Results and Discussion

Graph 1 shows the risk management model. Individually or cumulatively, threats, i.e., harmful effects as entomological damage, diseases, inadequate management, can, more or less, affect the functions of the forest. The forest origin and purpose interact with the functions of the forest. The consequence index is the product of the F-coefficient of the forest function with the corresponding R-coefficient of risk (threat). The Consequences Index (Ci) regarding the source of possible threats and adverse effects serves for risk analysis and determines management decisions and necessary measures. These measures can be preventive, preventive curative, and remedial.



Graph 1: Risk management model

Table 5 shows a specific example of a high natural conifer stand, which is a special reserve of nature. The first column describes the forest by purpose and origin, as well as health status. The second column shows the ten basic threats ranked by importance. The third column shows risk identification for each specific threat. In the fourth column, according to the origin and purpose of the forest, the F-valorisation coefficients of the forest function for a high natural stand of conifers are determined. In the fifth column, the risk identification determines the R-coefficient of risk (threat). In column six, the consequence index (Ci) is the product of the F-coefficient of the forest function and the R-coefficient of risk. The sum of the

consequence index, as a summary index, represents the assessment of the consequence. Column seven summarizes the analysis of system vulnerability analysis according to the consequence index. The column eight shows the proposed measures.
Table 5 shows the model where the vulnerability of the system is high, i.e., the total consequence index (Ci) is 37,827. There is a forest drying primarily due to infection with heterobasidion, then a secondarily strong attack of bark beetles. The trees dry out both individually and in groups. There is a risk of fire and snowbreaks and other damages of snow on trees. As a possible measure to reduce the presence of phytopathological diseases, the suggestion is constant monitoring, removal of diseased trees, the setting of pheromone traps, and treatment of stumps with a means of protection/repellent.

Table 5: The example of a high natural conifer stand, which is a special reserve of nature by its purpose

Forest description	Basic threats - adverse effects	Risk coefficients (1-5)	F-valorisation coefficients (Table 2)	R-valorisation coefficients (Table 3)	Consequence index Ci=(F) x (R)	Risk analysis - vulnerability (Table 4)	Risk management-measures
1	2	3	4	5	6	7	8
High natural conifer stand. Special nature reserve. Forest drying. Heterobasidion infection primarily, then secondarily strong attack of bark beetles.	Entomological damage	5	8,670	0,961	8,332	Cumulative consequence indeks - high risk. ∑(Ci)= 37,827 Drying of trees individually and in groups. There is a danger of fire, but also further drying of the forest	Rehabilitation, removal of diseased trees, installation of pheromone traps, treatment of stumps with a means of protection in order to reduce the presence of phytopathological diseases, ie rot. Constant monitoring.
	Phytopathological phenomena - diseases	5	8,670	0,910	7,890		
	Illegal logging	3	8,670	0,476	4,127		
	Management risk	4	8,670	0,459	3,980		
	Snowbreaks and windbreaks	5	8,670	0,494	4,283		
	The impact of fire	5	8,670	0,410	3,555		
	The impact of water erosion	3	8,670	0,257	2,228		
	The impact of floods	1	8,670	0,057	0,494		
	The impact of agrocomplex	3	8,670	0,181	1,569		
	The impact of hunting	3	8,670	0,158	1,370		
	Total consequence indeks ∑ (Ci)				**37,827**		

The Environmental Law does not leave much room for maneuver in the special nature reserve. Perceiving the first degree of protection often as a permanently preserved condition is wrong. It is visible that the index of consequences for the source of possible threats "Management risk" is the fourth most important (Ci=3,980). The impact of the agro-complex, in this case, is indirect and characteristic of the conditions in Serbia, because negligent actions of citizens in the contact zone of agricultural and forest land can endanger forests. The consequence index of entomological damage is higher than the consequence index of phytopathological diseases. At first glance, this seems contradictory. However, at a given moment, secondary damage from bark beetles is dominant. When complying with the Law on Inspection Supervision, the risk will be estimated as high, which requires appropriate legal measures.
For the wood certifier, the data given in the example are indicative concerning the certification principles. Based on the above parameters, comparing individual consequence indices with the cumulative consequence index ∑ (Ci), the forest user or owner assesses and decides whether and to what extent forest certification is required. Using wood material in the local market, and/or the presence of risks such as fire or impact of other threatening factors may adversely affect the value of the forest and the decision on the certification of the wood material. Using the same parameters, if analyze forests that have production functions, the summary consequences index would be ∑ (Ci) = 32,635, i.e., they would have a medium level

of risk. It indicates that this model, which manipulates several different parameters in risk assessment, justifies its application.

Conclusions

Harmonizing legislation at the operational level with management functions in forestry through the process of risk management and the need to introduce wood certification is an imperative task. Forest management can meet modern challenges and make forest management environmentally friendly, socially acceptable, and economically sustainable if there is a method where the forest user or owner assesses, analyzes, and makes adequate decisions.

What is specific for the activity of forestry is its own connection to the certain space (object). Ten basic forest functions, for the forests exposed to threats, i.e., possible risks, have been formulated. Forest functions interactively link to the spatial division according to the origin and purpose of the forest. Ten basic threats that can cause adverse consequences in the business and the conduct of forestry entities have been identified. The method is compatible with possible use in geographic information systems. The presentation of the results processed by this method is available in both a graphical and analytical form. In the risk management process, use interactive matrices and analyze 140 parameters. This method can be used effectively in the valorisation of risks in continental parts of Southeast Europe from the mountain, upland, and lowland forests. For forests in the Mediterranean and sub-Mediterranean area, i.e., alpine and subalpine, it would be necessary to make an adequate model, using the above principles

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EFFECT OF ACORN SIZE ON CONTENT OF PHOTOSYNTHETIC PIGMENTS IN LEAVES OF ONE-YEAR-OLD NORTHERN RED OAK (Quercus rubra L.) SEEDLINGS

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Abstract

The research objective was to examine effect of acorn size on content of photosynthetic pigments in leaves of one-year-old Northern red oak (*Quercus rubra* L.) seedlings. The seedlings have been produced from acorns which have been classified in three groups according to size. The content of the following photosynthetic pigments was examined: chlorophyll a, chlorophyll b and carotenoids. Leaf sampling was carried out in the mid-vegetation. The highest average value of chlorophyll a (0.639 mg/g) was determined in seedlings produced from the smallest acorns (group 1), and the lowest (0.544 mg/g) in seedlings produced from the largest acorns (group 3). The highest average values of chlorophyll b (0.465 mg/g) and carotenoids (0.510 mg/g) were determined in seedlings produced from medium-sized acorns (group 2). The lowest average values of chlorophyll b (0.369 mg/g) and carotenoids (0.188 mg/g) were determined in seedlings produced from the largest acorns (group 3). The strongest dependence was determined between carotenoids and chlorophyll b (R^2=0.9434) and the weakest between carotenoids and chlorophyll a (R^2 =0.0003). The studies have shown that the content of photosynthetic pigments in leaves of one-year-old Northern red oak varies depending on the size of acorns used for seedling production. Based on the obtained results, it can be concluded that during the selection of mother trees for the reproductive material production, attention should be paid to the size of acorns, and consequently the intensity of photosynthesis, which will later have a significant impact on seedling growth elements.

Key words: *Northern red oak, photosynthetic pigments, acorn, one-year-old seedlings.*

Introduction

Photosynthetic pigments are extremely important in the process of photosynthesis because their quantity and quality play an important role in the assimilation of plants. The importance of photosynthetic pigments is reflected in the absorption of sunlight necessary for the process of photosynthesis in plants. Chlorophylls are the primary pigments of photosynthesis. Chlorophyll a is the most common pigment in plants, it absorbs sunlight in wavelengths of 430 nm (blue) and 662 nm (red), and strongly reflects green light, so it seems green to us. Chlorophyll b has a similar structure as chlorophyll a, absorbing sunlight in wavelengths of 453 nm and 642 nm. Carotenoids reflect yellow, orange or red part of the spectrum, absorbing sunlight in wavelengths of 460 nm and 550 nm, respectively.

The ability to absorb sunlight of specific wavelengths is much more important for the process of photosynthesis than the ability to reflect a part of the sunlight (Popović et al., 2015a). Due to the various sizes and shapes, as well as the different content of pigments, the leaves have a different ability to absorb visible sunlight and convert it into the energy of chemical bonds. (Čivić et al., 2003). Foliar concentration of the main photosynthetic pigments chlorophyll a

and b is considered to be a bioindicator of the total primary biomass production (Gitelson, A., Merzlyak, M. N., 1994).

As the content of the photosynthetic pigments, in addition to the conversion of sunlight energy into organically rich compounds, affects the dominant coloration of higher plants, increased adaptability of trees and stands, and is important as a raw material for the pharmaceutical industry, the acquiring knowledge on variability of pigments within the species is increasingly significant (Mataruga et al. 2000). Determining the content of photosynthetic pigments in leaves is being justified for the reason that the intensity of photosynthesis largely depends on it, and consequently, the intensity of photosynthesis significantly affects the growth and development of seedlings.

The objective of research activities in this paper was to determine effect of size of acorns used for the seedling production on content and variability of photosynthetic pigments in leaves of one-year-old Northern red oak (*Quercus rubra* L.) seedlings.

Material and Method

Northern red oak seedlings were produced from seeds collected in a seed stand RS-2-2-qru-00-217 FMU Belgrade (Serbia). After collection, the acorns were dried to moisture content of 35% and stored at 3ºC.

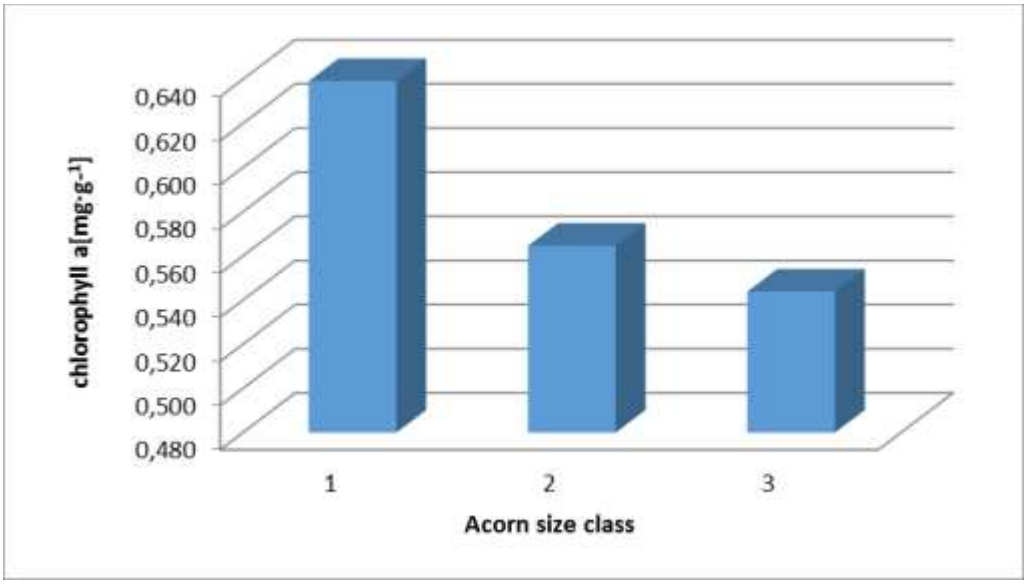
In order to determine the criteria for acorn classification into size classes, the measurements of weight, length and diameter were performed on a sample of 300 acorns before sowing. The measurements of the acorn length and diameter were performed using a vernier calliper with an accuracy of 0.01 mm, and the weight using an electronic scale with an accuracy of 0.01 g. After the measurement, the acorns were classified into three classes based on weight: small acorns ($\leq$ 5.50 g), medium-sized acorns (from 5.51 g to 7.00 g), and large acorns ($\geq$ 7.01 g).

The trial was set up in three replicates (all three acorn size classes were represented in each replicate) in the seedling nursery of the Institute of Forestry in Belgrade (Serbia). Three hundred acorns (3x100) were sown per each acorn size class. The hotbed 9x1 m was divided into 9 equal fields 1x1 m. One hundred acorns were sown in each field at a distance of 10x10 cm. After sowing, the seeds were covered with about 3-cm-thick layer of substrate.

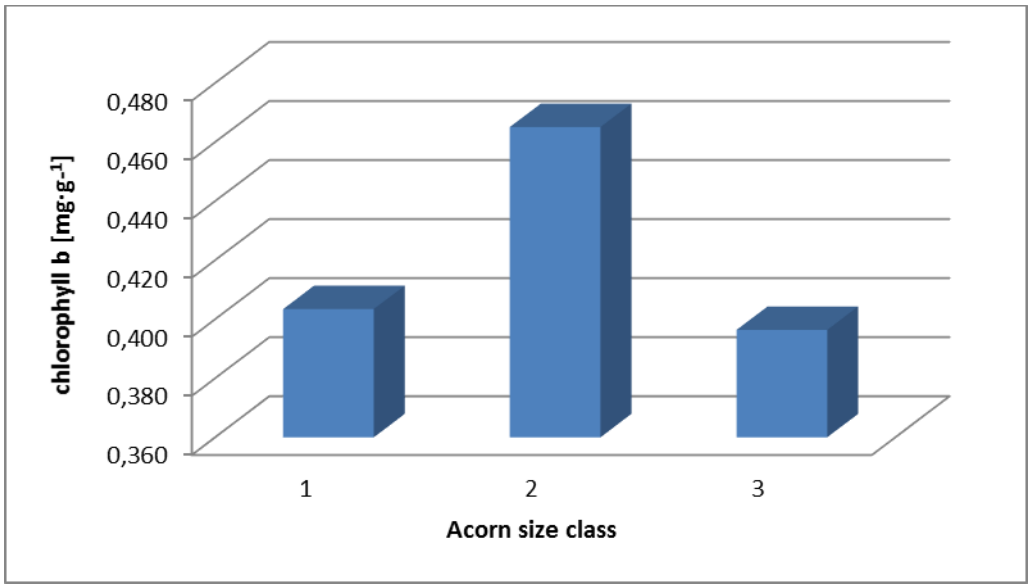
The leaf sampling from seedlings was conducted in the last week of August. A sample of 1 g was homogenized using a mortar and pestle. Two grams of quartz sand was added in the mortar before mechanical grinding for better sample homogenization. The paste was treated with 15 ml of 80% acetone for 3 minutes. One mg of $MgCO_3$ was added to this mixture in order to prevent acidification of the solution. The resulting green solution was applied by a small glass rod on a glass filter and thereafter, filtered into the vacuum test tube using a water spray vacuum pump. The resulting filtrate was the pigment extract which was transferred from the test tube to the regular 25 ml vessel and supplemented with 80% acetone to the line. The obtained extract had to be diluted in order to perform reading in a spectrophotometer. One ml of the obtained extract was taken with a pipette, after which 9 ml of acetone was added into it and then it was transferred into the test tube. Thus prepared extract was poured into the cuvette and read on the spectrophotometer, the absorption was in the wavelengths of 662, 644 and 440 nm. The formula of Holm and Wetsttein was applied to calculate the concentration of the pigment in the extract in mg / dm^3. Preparing and reading on the spectrophotometer as well as calculating were performed using standard methods (Oljača, R., Srdić, M., 2005). The effect of acorn size on the content of photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids) of one-year-old seedlings was examined using the one-way analysis of variance. Statistical analyzes were performed with the Statgraph 7 software package (StatSoft, Inc. 2004).

Results and Discussion

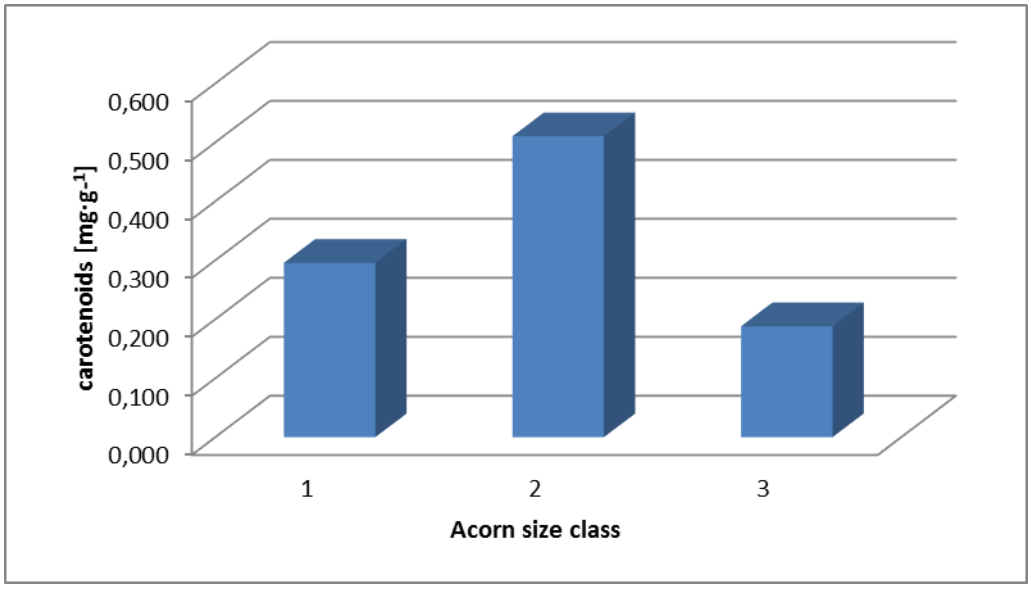
The content of photosynthetic pigments in the leaves of one-year-old Northern red oak seedlings by acorn size classes is presented in Graphs 1, 2, 3. Based on the presented results, it can be concluded that the content of photosynthetic pigments varies depending on the acorn size class used for seedling production. The highest average value of chlorophyll a (0.639 mg/g) was found in the seedlings produced from the smallest acorns (group 1), and the lowest (0.544 mg/g) in the seedlings produced from the largest acorns (group 3). The highest average values of chlorophyll b (0.465 mg/g) and carotenoids (0.510 mg/g) were found in the seedlings produced from medium-sized acorns (group 2). The lowest average values of chlorophyll b (0.369 mg/g) and carotenoids (0.188 mg/g) were found in the seedlings produced from the largest acorns (group 3).



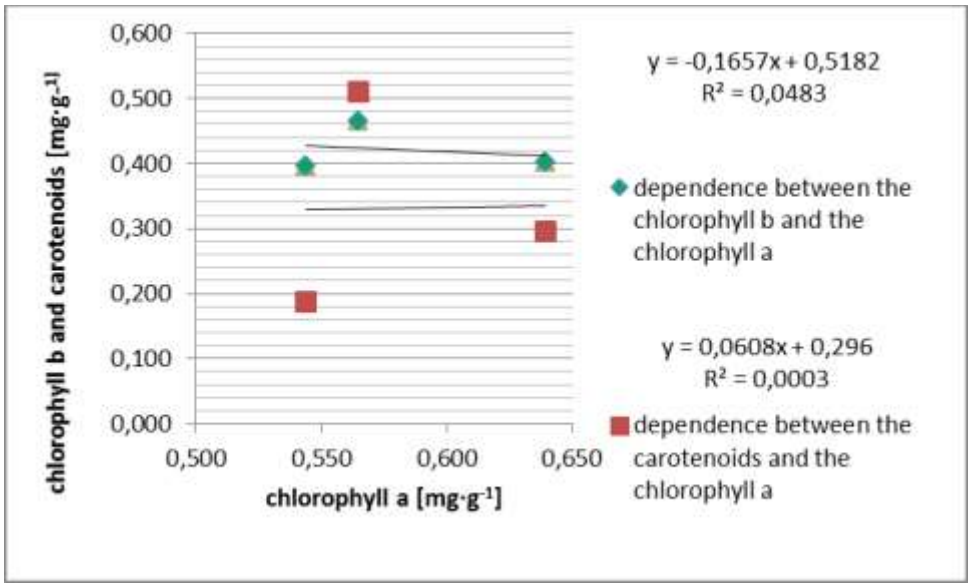
Graph 1. Content of chlorophyll a in leaves of Northern red oak seedlings by acorn size classes



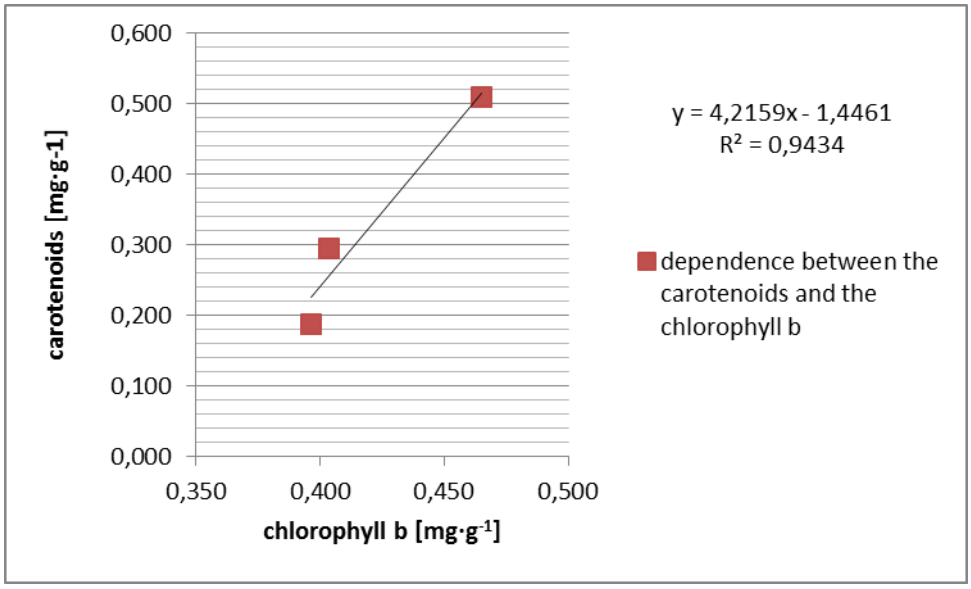
Graph 2. Content of chlorophyll b in leaves of Northern red oak seedlings by acorn size classes



Graph 3. Content of carotenoids in leaves of Northern red oak seedlings by acorn size classes



Graph 4. Dependence of the content of chlorophyll b and carotenoids on the content of chlorophyll a



Graph 5. Dependence of the content of carotenoids on the content of chlorophyll b

The strongest dependence was determined between carotenoids and chlorophyll b (R^2 =0. 9434) and the weakest between carotenoids and chlorophyll a (R^2 =0.0003) - Graphs 4 and 5. The effect of the acorn size class on the content of photosynthetic pigments was verified by the one-way analysis of variance. Based on the obtained results (Table 1), it can be concluded that the average values of the content of photosynthetic pigments statistically significantly differ. Considering that the seedlings were grown in uniform environmental conditions, the determined differences can be attributed to the effect of the acorn size classes used for the seedlings production.

Table 1. Results of one-way analysis of variance of the effect of the acorn size class on the content of photosynthetic pigments

Photosynthetic pigments	Acorn size classes			P- Value
	I	II	III	
Chlorophyll a	0.639[b]	0.565[a]	0.544[a]	0.0013
Chlorophyll b	0.403[a]	0.465[b]	0.396[a]	0.0051
Carotenoids	0.296[a]	0.510[a]	0.188[a]	0.0002

Foliar concentration of the major photosynthetic pigments chlorophyll a and b is considered a bioindicator of total primary biomass production (Gitelson, A., Merzlyak, M. N., 1994). For the needs of nursery production, it is very important to select the right source of reproductive material. This will ensure the production of quality seedlings, and thus significantly reduce unnecessary costs. Studies on the effect of seed size have shown that it is necessary to perform calibration in order to ensure quality production. The size of seeds used for the seedling production affects greatly on seedling quality and morphological parameters of one-year-old Northern red oak seedlings (Popović, et al. 2015b). A strong correlation between the acorn size and the content of reserve nutrients was determined in species of the genus Quercus (Tripathi, R.S., Khan, M.L., 1990). Studies carried out on some species of European and American oaks have shown that the acorn size is in a positive corelation with development of the aboveground part of the seedling and the higher root biomass (Tripathi, RS, Khan, ML, 1990; Matić, S. et al. 1996; Bonfil, C., 1998; Roth, V. et al. 2009; Ivanković, M. et al., 2011).

Conclusion

Based on the obtained results, it can be concluded that the content of photosynthetic pigments in the leaves of one-year-old Northern red oak seedlings varies depending on the acorn size class used for the seedling production. There is a possibility of increasing the quality of one-year-old Northern red oak (*Quercus rubra* L.) seedlings if acorns of appropriate size are used. The positive effect of acorn size on the content of photosynthetic pigments was significant. The seedlings produced from the medium-sized acorns had significantly higher average values of the photosynthetic pigments' content than the seedlings produced from the smallest and largest acorns which statistically significantly differ.

For a more comprehensive understanding of the importance of knowing the pigment content, it would be good to determine their content in different periods of vegetation, as well as the interdependence of pigment content and seedling growth elements.

Acknowledgements

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PSEUDOTSUGA MENZIESII (MIRB.) FRANCO IN THE NORTHWESTERN OF THE SPAIN: WOOD PROPERTIES, PRODUCTION AND FINAL CUT

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Abstract

The Oregon pine wood is of excellent quality and it is imported from North America. Afforestation by this species carried out in northern Spain, Galicia, Asturias and other regions; in many European countries is considered very important because it produce wood of equal or superior quality to the imported one. Oregon pine has slightly orange heartwood and clear sapwood. The growth rings are particularly visible, and with a proper forestry are very regular. Their density is higher than in most conifers of commercial interest, 0.48 gr.cm^{-3}. The wood has excellent technological qualities, even when the tree has grown quickly. Its major defect is the presence of knots, which are usually alive and so healthy. Pruning is essential to allow the tree development. The wood production that could be obtained depends on the site quality, the material and the repopulation techniques, and the cultural treatments. The French production tables of the Massif Central consider 3 quality classes with maximum production ranging from 15 to 23 m^{3}ha^{-1}year^{-1}. In Galicia and Asturias, growth studies have been carried out to compare with these tables, obtaining the following results: i) in the repopulations carried out in abandoned agricultural lands and with intensive cultural cares in Galicia, the quality far exceeds the best French and the production, if the density –number of trees per hectare– is high, reaches great values; the maximum production would be between 14 and 23 m^{3}ha^{-1}year^{-1}; ii) the researches carried out in Asturias show lower results due to that most of the stands of a certain age be correspond to forests repopulated by the Administration on poor quality soils. In any case, the production would be between 12.5 and 21.4 m^{3}ha^{-1}year^{-1}.

Keywords: *Pinaceae, Oregon pine, Wood features, Growth, Rotation.*

Introduction

Pseudotsuga menziesii (Mirb.) Franco, Douglas-fir, is a native conifer from North America that was brought to Europe in the 19th century. The first seeds were introduced by David Douglas in 1827 and planted at Dropmore Park, UK (Da Ronch *et al.*, 2016). In western North America, grows in an area of over 20 million hectares within a wide climate range generally quite dry summer. There are two subspecies: the coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) occurs from British Columbia southward along the Pacific Coast to central California; and the Rocky Mountain Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) occurs from central British Columbia along the Rocky Mountains into the central Mexico Mountains (Eckenwalder, 2009). Oregon pine has been developed as an important economic species because of their rapid growth and wood quality (Isaac-Renton *et al.*, 2014). In its origin area reaches over 100 m in height and a cylindrical stem up to 4 m in diameter, living more than 1300 years. It has slightly orange heartwood and clear sapwood (Praciak *et al.*, 2013). Their needle-like leaves are not sheathed and are 2-3.5 cm long (Figure 1) (Eckenwalder, 2009; Da Ronch *et al.*, 2016). Their density is higher than in most conifer species of trading interest, 480 kg.m^{-3}, and the wood has excellent technological qualities (Spiecker *et al.*, 2019). In the European countries where was established and now occurs in a

large area by afforestation, is one of the fastest growing trees. It prefers deep, moist, and well-drained soils, at mid-altitudes with an annual rainfall over 800 mm (Eckhart *et al.*, 2019).



Figure 1. Douglas-fir, botanical characteristics (cones and needle-shaped leaves) (Source: https://www.arbolesornamentales.es/Pseudotsugamenziesii.htm [Accessed 3th July 2019])

Spanish first plantations were ornamental, and in Galicia have preserved trees dating from the nineteenth century. In the Iberian northwest, its use during the afforestation period of the Forest Heritage was limited; the oldest stands have no more than 40 years. According to the available data, Douglas-fir occupies more than 800,000 ha in Europe, of which 75% are in France and Germany (Eckhart *et al.*, 2019). Regarding Galicia, an area of more than 70,000 ha is estimated (Galicia Government, 2018). The great growth rates and unique properties of wood, together a scarce pests and diseases are factors that have promoted their success and spread in Europe (Schmid *et al.*, 2014). Most stands of Asturias and Galicia are located on abandoned agricultural land of private property and often on low quality soils, especially in Asturias. Despite this, the growth can reach 19 $m^3ha^{-1}year^{-1}$. Pruning is essential to allow the right development of the tree because the wood major defect is the presence of knots (Figure 2). It is considered as an option to increase productivity and to adapt European forests to climate change (Spiecker *et al.* 2019). The objectives were: i) to study the wood properties, growth and production and; ii) to analyse the effect of forestry over the final cutting age.



Figure 2. Oregon pine (visible growth rings) (Source: https://www.wood-database.com/douglas-fir/ [Accessed 3th July 2019])

Material and Methods

Wood properties, growth and production

Galicia Forest Plan elaborated in 1992 through a planning of 40 years, included the afforestation of about 70,000 ha of Oregon pine; the most of which would be located in the eastern provinces, Lugo (34,000 ha) and Ourense (23,000 ha), and the rest in the Atlantic provinces, A Coruña and Pontevedra. This Plan was revised and modified in 2018 and some changes were made in the determination of the area occupied by the tree species. However, it is observed that the current area occupied by the Oregon pine differs only between 5% and 10% less than expected at the end of the plan, i.e. in 2032 (Galicia Government, 2018).

Through appropriate forestry treatments, Douglas-fir produces wood of much better quality than other conifers used today commercially in Spain. The growth and production is exceptional and its requirement less than most hardwoods (Praciak *et al.*, 2013).

Growth conditions and forest management practices influence the physical and mechanical characteristics of wood, bringing variations of its properties. As a result, although wood from centuries-old North American Douglas-fir has remarkable technological properties, the resource produced nowadays in second-growth American forests, as well as in areas where Douglas-fir has been introduced, may be very different (Henin *et al.*, 2019). In Europe, where Douglas-fir plantations developed, growth rate is considerably higher than that observed in old-growth forests and, for technical and financial reasons, rotations rarely exceed 100 years while cutting-diameter is generally below 70 cm (Kohnle *et al.*, 2019)

Forest management of Oregon pine stands in the study area

The French production tables of the Massif Central contemplate three quality classes with maximum production ranging from 15 to 23 $m^3ha^{-1}year^{-1}$. In Galicia and Asturias, growth studies have been carried out to compare with these tables, finding the following results: i) In the afforestation carried out in abandoned agricultural lands with intensive cultural cares in Galicia, the quality far exceeds the best French, and the production, if the density is high, reaches great values. Generally, the maximum production interval would be between 14 and 23 $m^3ha^{-1}year^{-1}$; ii) The researches carried out in Asturias show lower results due to that most of the stands of a certain age be correspond to forests repopulated by the Administration on poor quality soils. In any case, the production would be between 12.5 and 21.4 $m^3ha^{-1}year^{-1}$. (Solla *et al.*, 2006; López-Sánchez and Rodriguez-Soalleiro, 2009).

Results and Discussion

Properties of the wood, growth and production of Oregon pine

Douglas-fir heartwood presents a light brown to salmon colour, with red and yellow hues; sapwood is whitish to yellowish. Even though the species is known for its outstanding productivity, growth ring width of Douglas-fir varies significantly depending: i) the development conditions, ii) the forestry practices applied to the stands, iii) the genetic endowment of the trees, iv) age and the natural-social position within the stand, v) the crown development, as well as, vi) the wood nature (juvenile or mature). Depending on all these factors, the average ring width at breast height on a whole radius is generally comprised between 2 mm and 7 mm in European Douglas-fir trees. However, in plantations with extremely low tree density, the annual radial increment of young trees may exceed 10 mm during several years (Henin *et al.*, 2019).

According to large samples gathered throughout different Europe countries, the average density of Douglas-fir wood is about 500 $kg.m^{-3}$ at 12% moisture content. This property does not appreciably differ between sapwood and heartwood. Inversely, for a given ring width,

mature wood is about 10% weightier than juvenile wood. Nevertheless, the highest density heterogeneity is observed within annual rings, where density rises from less than 300 $kg.m^{-3}$ in earlywood to more than 900 or even 1,000 $kg.m^{-3}$ in latewood.
Today, Oregon pine planting is habitually discussed in relation to the adaptation of forest management to scenario of climate change. Through climate change situation expecting, it may be as a possible tree species even in those countries where the wood market for this tree species is not yet started (Hasenauer *et al.*, 2016; Eckhart *et al.*, 2019).

Effect of forestry treatments over the age of final cutting of Oregon pine

As I mentioned above, one of the exceptional characteristics of Douglas-fir is the species' great growth potential. For example, in France has an annual grow of 14.8 $m^3.ha^{-1}$, in Germany shows an average annual grow of 18.9 $m^3.ha^{-1}$. Despite these outstanding growth data, in Galicia the maximum production range would go from 14 to 23 $m^3ha^{-1}year^{-1}$ (López-Sánchez and Rodriguez-Soalleiro, 2009). So to achievement successfully the significant growth potential of Douglas-fir, forestry treatments has to address the following aspects (Kohnle *et al.*, 2019):

- Description of the production aims to be reached (e.g. timber dimension, quality, possible uses, and increase in added value).
- Establishment of site-adapted, healthy and strongly growing stands.
- Forestry treatments to optimize the age of final cutting of Oregon pine (e.g., spacing, thinning, pruning, even or uneven structure) that achieve an optimum between the characteristics of (diameter) growth speed, quality development and associated potential risks (e.g. storm damage).
- Control/maintenance of the appropriate mixed species.

In numerous Douglas-fir stands in the Northwest of Spain there are almost total absence of forestry treatments, so the density of trees is very high. The presence of many dominated trees and dead, which is a source of pest proliferation, is common. If not pruned, Oregon pines have branches from the base, which will have profuse knots and lose quality.
Then as Oregon pine is a species capable of producing high quality wood, the applied forestry must be guided to obtain that product. To achieve this, it will be necessary to prune, so that wood is formed free of knots, and make thinning, to eliminate branchy trees, forked or low vitality. The main goal would be the final cut was between 50 and 70 years (López-Sánchez and Rodriguez-Soalleiro, 2009).

Conclusions

Within its area of origin, Douglas-fir occupies a great area across a huge climatic range that in general features rather dry summers. From the end of the 19th century was introduced in Europe as an ornamental tree, later was established at a progressive rate in several European countries. Now Oregon pine is the second most abundant non-native tree species in European forests where it covers more than 800,000 hectares. The largest area of Douglas-fir is in France, followed by Germany where it has quickly become the most widespread non-native tree species. In other European countries, Douglas-fir is still growing to a significant extent, e.g. in Spain there is 70,000 hectares of afforestation. The main reason for growing Douglas-fir is its high productivity and the excellent wood properties. The average density of Douglas-fir wood is approximately 500 $kg.m^{-3}$ at 12% moisture content, without any appreciably differ between sapwood and heartwood. Then as Oregon pine is a species capable of producing high quality wood, the forestry must be guided to obtain it. So it will be necessary to prune, so that wood is formed free of knots. The goal would be the final cut was between 50 and 70 years.

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MIXED FORESTS OF BEECH AND SESSILE OAK (Querco petraeae-Fagetum moesiacae Glišić 1971) IN KOSMAJ PROTECTED AREA (SERBIA) – FLORISTIC AND EDAPHIC CHARACTERISTICS

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Abstract

The paper presents floristic and edaphic characteristics of a mixed forest community of submontane beech and sessile oak (*Querco petraeae – Fagetum moesiacae* Glišić 1971.) in the protected area of Kosmaj (Serbia). The vegetation of the small mountain massif of Kosmaj is composed of forest plant communities whose edifiers are different species of oaks and beech. In the investigated area, this community occurs on calcareous-silicate rocks. The following types of soil were determined: ranker, eutric cambisol, and luvisol. The floristic composition of the community is diverse. It includes thermophilic and mesothermal oak species, but also mesophilic species of beech forests. Based on the floristic composition and environmental conditions, the following two sub-associations were determined – *typicum* and *caricetosum pilosae*. In the spectrum of floral elements, plants of the Central European range type are most commonly represented, amounting to 40%. The study of the plant life forms shows that phanerophytes are the dominant class with 41%, followed by hemicryptophytes (28%). According to the indicator values of plants, the community of beech and sessile oak is mesophilic in terms of humidity, neutrophilic-basophilic in terms of acidity, mesotrophic in terms of soil nitrogen supply, sciophilic-semisciophilic regarding light, and mesothermal-thermophilic in terms of heat. Given that a large part of Kosmaj has been declared a landscape of outstanding features with significant natural, bio-ecological, and aesthetic values, research of vegetation can serve as a reliable guideline in the management of this protected area aimed at preserving and improving its biological diversity.

Keywords: *Kosmaj, beech-sessile oak forests, spectrum of floral elements, plant life forms, Serbia.*

Introduction

Beech forests are the most widespread forests in Serbia and cover 660400 ha or 29.4% of the total forested area (Banković *et al.*, 2009). Given its wide range of distribution in Serbia, it is understandable that beech is found in markedly different environmental conditions, i.e., different pure or mixed communities. Compared to Central European forests, the beech forests in Serbia are more complex and more diverse (Karadžić, 2018).

Mixed beech and sessile oak forest – *Querco petraeae-Fagetum moesiacae* Glišić 1971. is an intermediate forest community between montane beech forests and monodominant sessile oak or mixed sessile oak-hornbeam forests. It is characterized by greater floristic diversity and ground flora coverage compared to other montane beech forests (Tomić and Rakonjac, 2013).

In Serbia, it has been described on Boranja (Glišić, 1971), Fruška Gora (Jović *et al.*, 1989), Miroč (Cvjetićanin *et al.*, 2013), NP "Djerdap" (Cvjetićanin, 2013). The community of Balkan sessile oak and beech has been recorded on Goč, Suvobor, and Zlatibor, where it occurs on serpentinite (Cvjetićanin, 1999).

The aim of this research was to present edaphic characteristics, floristic composition and ecological conditions of beech-oak plant community (*Querco petraeae – Fagetum moesiacae* Glišić 1971.) in the protected area of Kosmaj (Serbia). Bearing in mind that most of Mt. Kosmaj has been declared a landscape of outstanding features, with significant natural, biological, ecological, aesthetic and cultural-historical values, the research of vegetation in this area can serve as a starting point for the planning of silvicultural needs as important factors in the provision of special benefits these forests have.

Material and Methods

Kosmaj is a low (626 m) and relatively small mountain, 40 km southeast of the city of Belgrade in Serbia. Kosmaj Mountain has been under an optimum model of protection since 2005 when it was declared a landscape of exceptional features. The total surface area of this protected area is 3514,50 ha. Over 70% of the mountain is under forest dominated by coppice oak and beech stands.

The study of the floristic composition of the association was based on ten relevés, made following the Braun-Blanquet method (Braun-Blanquet, 1964). The spectrum of floral elements was determined according to the systematization of geo-floristic elements by Gajić (1980), the spectrum of life forms according to the Method of Kojić *et al.* (1997), and the indicator values of plants and ecological optimums were determined using the Method of Kojić *et al.* (1997). The syntaxonomic ranks here used were harmonized with the International Code of Phytosociological Nomenclature (Weber *et al.*, 2000) by Tomić and Rakonjac (2013).

Soil types were determined at all sites where relevés were collected (May – July 2016). A soil profile was dug at representative sites to determine the type of soil and take disturbed soil samples for laboratory tests. Laboratory soil tests were performed using the standard methodology.

Results and Discussion

Mixed beech and sessile oak forests – *Querco petraeae-Fagetum moesiacae* Glišić 1971. on Kosmaj occur in a wide range of altitudes (relevés range from 373 to 585 m a.s.l.), cold northern aspects (northern, north-western, and north-eastern) and slopes of 16° – 26°. In the study area, this community grows on ranker, eutric cambisol and luvisol over calcareous-silicate rocks.

The investigated humus-silicate soil (ranker) belongs to the eutric subtype and regolith variety. The depth of the soil type is 50 cm. In terms of texture, it is classified as loam. The soil is permeable to water and aerated, but also quite skeletal. It has a high content of total humus. The nitrogen content is quite high, and the C/N ratio is narrow. The total cation adsorption is high, resulting from the high content of humus.

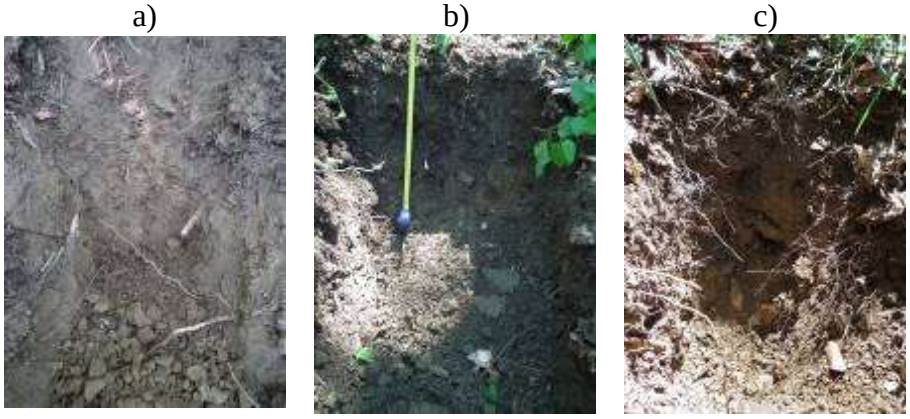


Figure 1. Soil types: a) Eutric cambisol b) Ranker c) Luvisol

The investigated profile of eutric cambisol is 60 cm deep and skeletal. Regarding the texture, this soil type belongs to the class of loam at all depths. The humus-accumulative horizon has a large amount of humus, and the cambic horizon is poor in humus. The ratio of carbon to nitrogen is narrow, which indicates conditions favourable for biochemical processes of organic matter decomposition. The degree of base saturation exceeds 50% of the total adsorption capacity, which classifies these soils as eutric.

Ilimerized soils (luvisols) are often present in forest communities with beech as an edifying species and make highly productive habitats for beech (Knežević, 2003). The depth of the investigated luvisol is 60 cm. Rock fragments are scarcely present, mainly in the deeper parts of the illuvial horizon, while the surface parts of the profile lack skeleton. The soil is texture differentiated. The surface layers belong to sandy loam classes and the deepest layers to loams. Regarding the content of humus, the humus-accumulative horizon is quite humus-rich. The eluvial horizon is poor, and the illuvial horizon very poor in humus. The sum of the adsorbed base cations is low.

The floristic composition of the community is diverse. It includes thermophilic and mesothermal oak species, but also mesophilic species of beech forests. The total number of plant species found in this community is 78 (Table 1).

Of the edifying species in the tree layer, beech (*Fagus sylvatica* L.) exceeds sessile oak (*Quercus petraea* (Matt.) Liebl) both in terms of the abundance and cover. The shrub layer is either completely absent or poorly developed, with the following ten species occurring singly in it: *Crataegus monogyna* Jacq., *Fagus sylvatica* L., *Sambucus nigra* L., *Cornus mas* L., *Acer pseudoplatanus* L., *Fraxinus excelsior* L., *Corylus avellana* L., *Crataegus nigra* Waldst. et Kit., *Malus silvestris* (L.) Mill. The cover of the ground flora layer ranges from 0.2 to 1.0. Mesophilic species of beech forests occur with the highest degree of presence: *Lamium galeobdolon* (L.) Crantz, *Carex sylvatica* Huds., *Dryopteris filix-mas* (L.) Schott and *Rubus hirtus* Waldst. & Kit.

Based on the floristic composition and site conditions, the community is divided into 2 sub-associations: *typicum* and *caricetosum silvaticae*. The *typicum* subassociation is rich in flora and makes a stable typical set. The *caricetosum silvaticae* subassociation is characterized by the presence of *Carex sylvatica* Huds. in the ground flora layer. Significant presence of species such as *Rubus hirtus* Waldst. & Kit. and *Alliaria officinalis* (M. Bieb.) Cavara & Grande in this community is caused by anthropogenic factor.

Table 1. Phytosociological table of *Querco petraeae - Fagetum moesiacae* Glišić 1971.

Association	*Querco petraeae - Fagetum moesiacae* Glišić 1971.										
Sub-association	*typicum*							*caricetum pilosae*			
Relevé number	1	2	3	4	5	6	7	8	9	10	Degree of presence
Altitude (m)	567	585	544	502	414	428	373	400	466	384	
Aspect	N	N	N	N-NW	N	N	N-NE	N-NE	N	E-NE	
Slope (°)	16	16	24	17	17	19	26	18	17	26	
Bedrock	calcareous-silicate rocks										
Soil	Ranker			Eutric cambisol				Luvisol		Eut.c.	
Tree layer											
Canopy	0,9	0,9	0,9	0.9	0,9	0,9	1,0	0,8	1,0	1,0	
Fagus sylvatica	4.4	4.4	5.5	4.4	3.1	4.4	3.4	4.2	4.4	4.4	V
Quercus petraea	2.1	2.1	1.1	3.1	2.1	2.1	1.1	1.1	2.1	1.1	V
Quercus cerris	+.1	1.1	+.1		1.1	1.1		1.1	+	+.1	IV
Acer campestre	1.1		+	+			2.1				II
Tilia tomentosa								1.2	+		I
Quercus farnetto		+						+.1			I
Populus tremula							+.1	+			I
Prunus avium					1.1				+		I
Tilia platyphyllos				2.1							I
Acer platanoides				1.1							I
Acer pseudoplatanus				1.1							I
Carpinus betulus					1.1						I
Shrub layer											
Canopy		0,1	0,1	0,1	0,1	0,1	0,1	0,1			
Crataegus monogyna				1.1	1.1			1.2			II
Fagus sylvatica			1.1	1.1			+.1				II
Sambucus nigra		1.1		1.1			+				II
Cornus mas		1.1			1.1						I
Acer pseudoplatanus				1.1	1.1						I
Fraxinus excelsior		1.1									I
Crataegus nigra		1.1									I
Corylus avellana					1.1						I
Malus sylvestris						+					I
Ground flora layer											
Coverage	0.3	1.0	0.9	1.0	0.9	0.6	0.2	0.2	0.7	0.2	
Lamium galeobdolon	2.3	5.5	4.5	5.5	5.5	2.2	2.3		3.3		IV
Carex sylvatica	+.2		2.2	1.2	2.3	+.2	2.2		2.2	+.2	IV
Dryopteris filix-mas	+.2	2.2	2.2	2.2	2.2	2.2			2.2		IV
Rubus hirtus			1.1	1.1	3.1	1.1	1.2	1.1	1.1		IV
Helleborus odorus	+	2.1		+.1	1.1	1.1		+			III
Cardamine bulbifera		1.2			3.1	4.4	2.1		3.1		III
Alliaria officinalis			1.3	2.1	2.1	2.1			1.1		III
Prunus avium	+				1.1			+.1	2.1	+	III
Circaea lutetiana	2.1			2.1		4.4	2.3				II
Asperula odorata	2.3		2.3	2.3					2.2		II
Hedera helix					1.1		1.1	1.1		+.1	II
Stachys silvatica		+.1			2.1	+.1	1.1				II
Viola hirta	2.1						1.1	+.1		+	II
Moehringia trinervia	1.2		1.2	+.2					1.2		II
Geranium robertianum	1.1		+.1	+.1	1.1						II
Quercus cerris					+		+.1	+.1		+	II
Carex pilosa								3.3	4.4	3.3	II
Fagus sylvatica							2.1	1.1		1.1	II
Viola sylvestris			1.1		1.1		1.2				II
Acer pseudoplatanus				2.1	2.1				+		II
Euphorbia amygdaloides			+		1.1			1.2			II
Fraxinus ornus	+.1						+	1.1			II
Ruscus hypoglossum	1.1		+		+.2					1.2	II

Viola odorata	+.1					1.1		1.1				II
Fragaria vesca				+.1			+.1				+	II
Sambucus nigra		+.1	1.1						+.1			II
Ajuga reptans					1.1		1.2					I
Galium silvaticum							1.1	1.2				I
Crataegus monogyna	+.1				1.1							I
Cornus mas	+.1				1.1							I
Lysimachia punctata	+							1.1				I
Mycelis muralis			+	+.1								I
Veronica montana						+.2			1.2			I
Tamus communis	+					1.1						I
Arum maculatum				+	+							I
Lilium martagon				+		+						I
Lathyrus vernus							+		+			I

Species present in one relevé only: *Carex pendula* 2.3 (5); *Athyrium filix femina* 2.2 (6); *Acer pseudoplatanus* 2.1 (4); *Allium ursinum* 2.1 (1); *Acer campestre* 2.1 (1); *Asarum europaeum* 1.2 (4); *Melica uniflora* 1.2 (8); *Calamintha vulgaris* 1.2 (8); *Acer platanoides* 1.1 (4); *Heracleum sphondylium* 1.1 (4); *Rubus canescens* 1.1 (1); *Ulmus minor* 1.1 (1); *Rosa canina* 1.1 (5); *Pteridium aquilinum* 1.1 (5); *Polygonatum multiflorum* 1.1 (5); *Polystichum setiferum* +.2 (2); *Ruscus aculeatus* +2. (10); *Fraxinus excelsior* +.1 (1); *Juglans regia* +.1 (6); *Populus tremula* +.1 (7); *Scrophularia nodosa* +.1 (8); *Lonicera caprifolium* +.1 (8); *Stachys alpine* + (3); *Cardamine impatiens* + (4); *Euonymus europaeus* + (1); *Clematis vitalba* + (1); *Lamium maculatum* + (1); *Ranunculus cassubicus* + (5); *Quercus petraea* + (5); *Polygonatum odoratum* + (6); *Urtica dioica* + (6); *Campanula patula* + (7); *Tilia tomentosa* + (7); *Campanula persicifolia* + (7); *Cephalanthera longifolia* + (10); *Prunus spinosa* + (10);

In the spectrum of floral elements, the most represented species are of the Central European range type (40%), followed by the species the Eurasian range type (20%) (Table 2).
Generally, mesophilic plants predominate in the community with 49% (Central European and Subatlantic range types). Plants of wide ecological amplitude follow with 25% (Eurasian and Cosmopolitan range types). Xerothermophilic plants account for 19% (Pontic, Sub-Mediterranean and Balkan range types), which indicates the presence of plant species of thermophilic oak forests. Circumpolar range types are represented with 5%, while the desert floral elements account for only 1%. A similar spectrum of range types was recorded for this community on Miroč (Cvjetićanin *et al.*, 2013).

Table 2. Spectrum of floral elements

Cumulative range types	Number of plants	Share (%)	
Pontic	5	6%	19%
Sub-Mediterranean	7	9%	
Balkan	3	4%	
Central European	31	40%	49%
Subatlantic	7	9%	
Desert	1	1%	1%
Eurasian	16	20%	25%
Cosmopolitan	4	5%	
Circumpolar	4	5%	5%
Total:	78	100%	100%

The dominant life forms in this community are phanerophytes with 41% (phanerophytes 27%, nanophanerophytes 13%, and phanerophytic lianas 1%), followed by hemicryptophytes (28%). The high share of geophytes (23%) indicates favourable soil conditions (moisture, structure, and depth of soil). The intermediate group of plants between therophytes and chamaephytes accounts for 5%, with chamaephytes having the same percentage of plants.

Table 3. Spectrum of life forms

Phanerophytes	Nanophanerophytes	Phanerophytic lianas	Herbaceous chamaephytes	Hemicryptophytes	Geophytes	Therophytes / Chamaephytes
27%	13%	1%	3%	28%	23%	5%
41%						

The average ecological indicator values of plants in this community are shown in Table 4. The community of *Querco petraeae-Fagetum moesiacae* Glišić 1971. is mesophilic in terms of humidity, neutrophilic-basophilic in terms of acidity, mesotrophic in terms of soil nitrogen supply, sciophilic-semisciophilic in terms of light, and mesothermal-thermophilic in terms of heat.

Table 4. Average ecological indicator values

Moisture	Soil reaction	Nitrogen	Light	Temperature
3.03	3.30	2.86	2.71	3.42

It can be concluded that mixed communities of beech and sessile oak occur in wet site conditions, with little light and in moderately warm conditions. The soils are neutral to slightly alkaline, moderately supplied with minerals.
Knowledge of ecology, i.e., relationships of forest trees to environmental conditions and biological species characteristics is of great importance in silviculture. It provides firm grounds on which stand silvicultural approaches and treatments can be determined and appropriate methods of natural regeneration and forest tending selected (Krstić, 2003).

Conclusions

Mixed communities of beech and sessile oak (*Querco petraeae-Fagetum moesiacae* Glišić 1971.) on Kosmaj grow on ranker, eutric cambisol, and luvisol over calcareous-silicate rocks. The floristic composition of this community is diverse, with a total of 78 plant species. They are classified into two sub-associations – *typicum* and *caricetosum pilosae*. The *typicum* sub-association is the most common variant. The sub-association of *caricetosum pilosae* is characterized by the presence of a differential species of hairy sedge (*Carex pilosa* Scop.) in the ground flora layer. Regarding the spectrum of floral elements, mesophilic plants predominate in this community with 49%, while the study of the spectrum of life forms indicates that the community is of phanerophytic-hemicryptophytic character with an increased share of geophytes. Indicator values of plants classify the community of beech and sessile oak as mesophilic in terms of humidity and neutrophilic-basophilic in terms of acidity. It is mesotrophic in terms of soil nitrogen supply, sciophilic-semisciophilic in terms of light, and mesothermal-thermophilic in terms of heat.

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EXAMINATION OF THE CONDITIONS OF FOREST SHELTERBELTS IN DISTRICT DOBRICH (NORTHEASTERN BULGARIA) USING GIS

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Abstract

The state forest protective belts established in the Dobrudzha plateau (Northeastern Bulgaria) in the early 50s of the last century have had an immense and lasting business and ecological impact. Apart from blocking winds and decreasing soil erosion, the shelterbelts keep soil moisture, and improve soil fertility and agricultural yields: thus supporting the sustainability of agricultural activity in the region. Shelterbelts have remained in the possession of the state even after Bulgaria's political transition to democracy; that ensured that protective belts (along with their beneficial functions) remained physically intact and at their original geographical locations. Nowadays shelterbelts' well-being is affected by property disputes, by illegal logging, and by poor agricultural management and maintenance of sanitary conditions. One way to decrease the effects of the latter problems is to introduce a state-of-the-art database software system for managing spatial data of shelterbelts. Using Geographic Information Systems (GIS) technology, we developed a unified information system for forestry protective belts based on data from Forest management plan of our chosen forest area (State Forest Enterprise "Dobrich", district Dobrich). We classified shelterbelts by type, by development scheme, by tree species and origin, by age, and by other indicators. We built database queries in GIS environment and obtained aggregated data on the shelterbelts from the territory of the SFE "Dobrich". Using automated algorithms, we identified potential sites of illegal activity and areas endangered by landslides and already affected by flooding or fire. The results of our analysis are the basis of adequate solutions for the protection of shelterbelts in the district. We have developed thematic and interactive maps that can be accessed via free GIS platforms. We have improved preparedness and response speed of forestry organs by integrating our data and tools into their operations. The end result is a boosted efficacy in the efforts to conserve forest protective belts.

Keywords: *GIS, forestry, protective forest belts*

Introduction

The use of agroforestry is a tradition in Bulgarian forestry and agriculture. Protective forest belts (PFBs) are an important type of agroforestry system, established more than 70 years ago, preserved and operating today in North-eastern Bulgaria, unique within Europe. They are designed to protect the soil, to improve the microclimate and to achieve other benefits for the environment (Marinov and Genova, 2008). These linear forest plantations are invaluable facilities for wildlife as a refuge for animal species and free-range animals. The belts have an exceptional economic effect for the protection of crops, agricultural production, and for increasing the yields of our farmers over 20-25% (Peev and Hinkov, 2000). These shelterbelts are forests with tree species such as poplar, acacia, willow, linden, and sycamore, and are located along ravines, canals, rivers, reservoirs, roads, fields, and meadows in agricultural areas, in order to stabilize river banks and reduce floods (Kachova, 2014).
There is a multitude of political attitudes, documents, and law-making, which encourage the development of various agroforestry systems.

The Forestry Act regulates and categorizes the agroforestry system of "protective forest belts", which are technically forests, but by their purpose and design are ameliorative tools in (Forestry Act, 2011). The Ministry of Agriculture, Foods, and Forestry is the authorized institution via Executive Forestry Agency (EFA) to manage and preserve PFBs and to facilitate activities for their restoration and maintenance.
The first PFBs were established in 1925 in Southern Dobrudzha. The entire system of PFBs in the region of Dobrich, Balchik, and General Toshevo (Decree of the Council of Ministers, 1951) was completed in the period 1951-56. The result is 785 km of PFBs in an area of 7385.3 ha (Stancheva *et al.*, 2015). Following 70 years of use, they need rehabilitation and reconstruction. Nowadays PFBs are forest territories with public property, frequently affected by illegal logging, poor management, and biotic and other factors of poor phytosanitary state. The aim of this research is to study the condition of protective forest belts through spatial analysis, thematic mapping, and updating of the data in a GIS environment.

Materials and Methods

Our study examines a forest territory with an area of 18,951.5 ha in the State Forestry Enterprise (SFE) "Dobrich" district Dobrich, North-eastern Bulgaria (Figure 1). We have developed a unified information system for protective forest belts with an area of 1,885.2 ha, based on control field data and inspections in 2019, and data from the Forest Management Plan (FMP) of SFE "Dobrich", effective as of 2015 (http://www.agrolesproject.com). The study used existing vector and attribute data for the protective forest belts, extracted from the FMP and National Information Forestry System (http://www.system.iag.bg/). Raster data includes freely available satellite data from Google Earth Pro and orthoimages of the territory from EFA.



Figure 1. Study area SFE „Dobrich"

Authors built a specialized database for the study area using "GISExplorer" (https://www.gisexplorer.eu/index.php). It contains vector and geocoded forest belts' attribute data (such as tree species, origin, age, property, and other indicators).

Results and Discussion

Authors propose the automated GIS method for analysis and creating of digital thematic maps as a step in assessing the state of the protective forest belts. As a result, the aggregated data, maps, and analyses are generated from the extended and specialized GIS database of the SFE "Dobrich". We prepare interactive and mobile maps of the study area for use in the field work forestry professionals. Existing spatial data has been verified and updated using GIS. The results of this study will ensure forest control and protection of forest belts.

Using the GISExplorer application, we obtained thematic maps of PFBs by various criteria: tree species, age, planned activities (type of felling), property type, and state of PFBs (figure 2). Database queries are constructed and executed via the GIS application extensions, which generates tables and figures containing aggregate data of PFBs area distribution (absolute values in hectares and percentages of total area) by tree species, age category, type of felling, agricultural category, type of property, state of PFBs (three-level scale) (Figure 2).

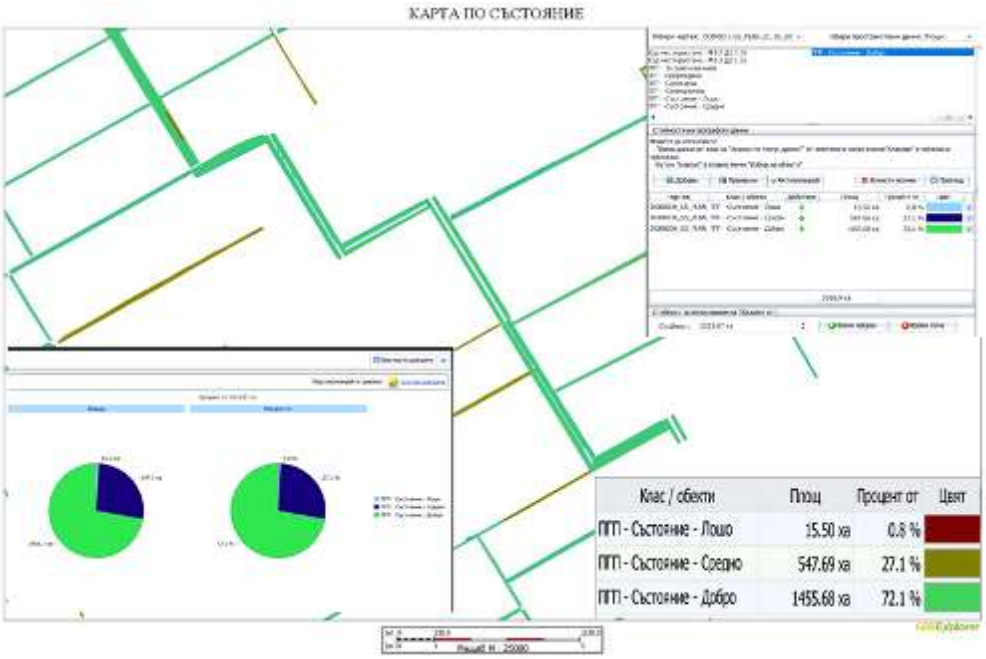


Figure 2. Part of the thematic map and area distribution by state of PFBs

Distribution of PFB by property type shows some presence of non-state property: 93.2% is state-owned, 5.4% is municipality-owned, and 1.4% is privately owned. Our analysis also enumerated the species diversity in the PFBs on the territory of SFE "Dobrich": the most widespread is the tree species *Quercus cerris* L. (32.8%), followed by *Gleditschia triacanthos* L. (21.2%), *Robinia pseudoacacia* L. (17.0%), *Fraxinus excelsior* L. (16.7%). The age of PFB is an important indicator in our study. The age distribution within the PFB is spread across seven classes: class 1 (1-10 years old) covers 22.1% of the PFB area; class 2 (11-20 years old) covers 14.5%; class 3 (21-30 years old) covers 3.6%; class 4 (31-40 years old) covers 0.6%; class 5 (41-50 years old) covers 1.5%; class 6 (51-60 years old) covers 56.9%; class 7 (61-70) covers 0.7%. The class with greatest area share is class 6, with ages 51 to 60 years old. The average age within the PFB is 37 years old. The overall sanitary state of the PFB is good; state can be one of three levels: good (72.1% of the PFB area), average (27.1%) and poor (0.8%).
In this study we apply technology to discover the incongruence between actual and digitally recorded state of PFBs. There are a number of problems which necessitate the new technical approach in assessing the state of PFBs (Asenova, 2018): issues in digital models of agricultural and forest areas; conflicts among property owners of agricultural land sharing borders with PFBs; the subpar conservation of forest stands in PFBs; the relatively old age of the forest stands within PFBs; and the errors made in the process of re-privatization of public land and the poor quality of land stewardship thereafter. All of the above contribute to the deterioration of forest stands in the forest areas to the point of inability to perform their protective role.

We procured data describing 14 such cases of incongruences while analyzing the territory of SFE "Dobrich" using our GIS tools. Some cases are inconsistencies in the geometrical properties (length, width, area) as well as specific characteristics such as type of territory, purpose, type of permanent use (TPU) of the land, and property type.

For every incongruence case, the vector data of the borders of sub-compartments (forest stands) and of real-estate properties are visualized in GIS alongside with their raster data (digital images overlaid in Google Earth Pro). We executed table query tables by forest subcompartment (sub-unit) and by property and aggregated those in new tables. Following analysis using GIS and Google Earth Pro images and on-site visits of every problematic sub-unit, we established there is a mismatch between the TPU and the actual use and state of the land.

For every case, we input the following data into the GISExplorer application: available vector data of the digital model layer "LESO", based on the Forest Management plan (FMP) data and the attribute data of the forest sub-units in the PFBs (Figure 3); the layer "Cadastre", based on the cadastre map (https://kais.cadastre.bg/bg/Map); data on the ownership of forest and agricultural real-estate properties; orthoimages and satellite imagery; and data from field GPS measurements. The results are presented in a common format laid out (see figures 3, 4, 5. and 6) below case #5 (used a sample case).

For example, in Case #5 (forest sub-compartment "2034:a" and cadastral unit № 77284.47.7, settlement Hitovo), due to the "evident fact errors" done during the restitution process, the actual forests in this forest belt have been restituted as "agricultural field". The layer "Cadastre" designates the land use as "field", but in reality the land of the PFB is covered by forest (Figure 4). According to the layer "LESO" the entire property is only forest (Figure 3). Judging from satellite images from the past 10 years, we also confirm that the area is indeed forest (Figure 5). It is necessary to update the attribute data in the cadastral map, according to the requirements of the Forestry Act.

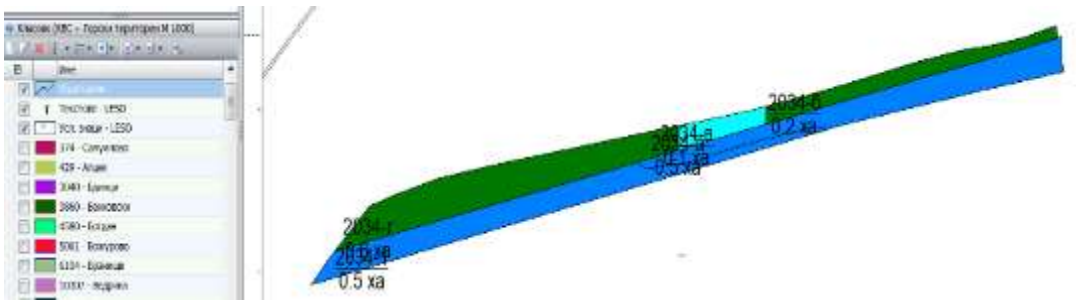


Figure 3. Vector data of subcompartment 2034:a and ownership according to the layer LESO

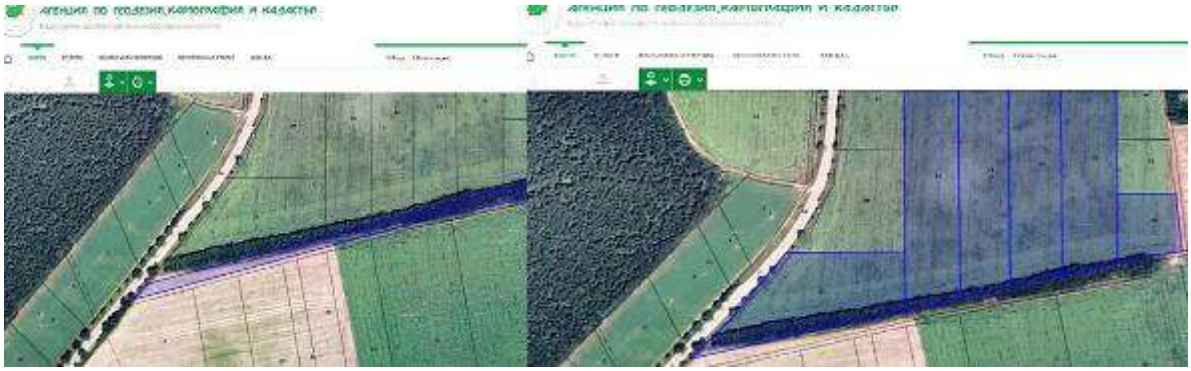


Figure 4. Data for a state-owned property (cadastre ID № 77284.47.7) (on the left) and its neighbouring properties (on the right) according to the layer Cadastre



Figure 5. Vector and raster data of a forest belt (private and state-owned) in Google Earth Pro

Землище	Отдел	Площ ха	Подотдел	Имот №	НТП	Собственост	Възраст	Площ на подотдела ха	Основен запас
Хитово	2034	1.8	2034 - а	77284.47.7	гора	дрзп	20изд	0.4	30
Хитово			2034 - б	77284.43.12	нива	фпзз	20изд	0.2	15
Хитово				77284.43.13					
Хитово				77284.43.14					
Хитово				77284.43.19					
Хитово			2034 - в	77284.43.15	нива	пгпф	20изд	0.1	5
Хитово			2034 - г	77284.43.21	нива	фпзз	20изд	0.6	40
Хитово			2034 - 1	77284.47.7	гора	дрзп	-	0.5	-
			ВСИЧКО			-	-	1.8	90

Figure 6. A sample of the attribute data of state-owned real estate № 77284.47 and forest belt sub-unit 2034:a

The aforementioned 14 cases of data incongruence contain a total of 75 forest stands - ПГП amounting to an area of 54.4 ha. The age of the latter forest stands varies from 3 to 60 years old. The most common age group is 60 years; it covers an area of 47.8 ha. The remaining age groups are: 3-year olds cover 0.7 ha, 12-years olds - 0.6 ha, 20-year olds - 1.6 ha, 50-year olds - 0.9 ha, and lastly, the non-wooded areas covers 2.8 ha. The area also contains 99 cadastre units (real-estate properties): the most which are individuals (57), followed by 25 state-owned and 17 municipal-owned properties.

The most commonly encountered inconsistencies are due to: incorrect mapping of PFBs; incorrectly recorded TPU data for the PFB; ownership disputes over PFBs in along shared borders between agricultural and forest territory. The various inconsistency cases can be fall into three different groups:

1. In PFBs there could be a number of different types of ownership (state, municipal, private/individual, and corporate) and not just state (public) ownership. According to the Forestry Act all PFBs are state-owned properties and the presence of any other type of ownership on the territory of a PFB must be clarified and corrected.
2. There are inconsistencies between PFB purpose, type of the territory and the rules for using the properties. This is due to the mapping inaccuracies in the initial forest planting phases and during later felling phases on the territory of PFBs. In most recent times, modern navigation and mobile GIS technologies help perform mapping with much greater precision and in shorter time and costing less.
3. PFBs are often subject to ownership disputes, because real estate properties tend to be finely and irregularly fragmented, while the shape of PFB parcels are almost linear with narrow widths, and lastly because other-than-state ownership has been allowed on the territory of PFBs. Errors are committed when mapping the contours of PFBs due systematic and sporadic errors while digitizing and due to mixing data from maps of different scales (1:5 000, 1:10 000, or smaller scale).

The reason real-estate property tends to be finely and irregularly fragmented is the incorrect in-place restitution of agricultural land that happens to be within existing PFBs as well as

illegal pursuit of owners of agriculture land to expand and cut into neighbouring PFB land and illegal use of PFB land not according to its original function and purpose.

Conclusions

Protective forest belts (PFBs) and their specific protective functions are an essential state resource with national agro-technical and ecological significance. It is of utmost importance to practice responsible management, conservation, and restoration of PFBs.

Our study of PFBs in SFE "Dobrich" using GIS has led us to the following conclusions and actions.

We propose GIS technology to be employed for the analysis of graphical and attribute data of forest areas, in order to increase the trustworthiness of data regarding the state of PFBs.

Using GIS we can achieve the automation of discovering and correcting "evident fact errors" and inconsistencies between real-life and digital models. Thus we can obtain real-time data updates and accurately reflect the changes happening in PFBs and their neighbour areas.

To improve monitoring of the state of PFBs, we recommend that specialist foresters more readily use GIS, freely available and open-source platforms, and mobile applications.

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http://www.agrolesproject.com

https://kais.cadastre.bg/bg/Map

https://www.gisexplorer.eu/index.php

http://www.system.iag.bg

POSSIBILITY OF ESSENTIAL OIL RATIONAL PRODUCTION FROM GREEN PARTS OF CONIFER TREES ON THE ROMANIJA MOUNTAIN (BOSNIA AND HERZEGOVINA)

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Abstract

Forests on the mountain Romanija (Entity of Republic of Srpska, Bosnia and Herzegovina) are rich in conifer species (*Abies alba, Picea abies, Pinus silvestris, Pinus nigra*) presenting the base of the growing stock with 134.000 m^3 of annual cut. After timber harvesting in forest there are side products as well needles, strobiles, offshoots and branches mid-diameter 1 – 4 cm that do not have any use, but they are actually the raw material for essential oil production. Using the survey, data were collected from the forest management unit „Romanija" Sokolac, public enterprise of forestry „Šume Republike Srpske". The questions referred to the basic data about the FMU and quantity of wood assortiment placed on the market selected by tree species. From green parts of conifer trees 2.000 tons per year (50% of total mass) in the period 2005-2014 and a middle yield coefficient at the beginning of vegetation and at the end of the growing season $YCeo_{\Delta}$ = 0,3725 cm^3 oil / 100 g it is possible to produce annually about 7.456 liters of high quality and market desired essential oil. The production is seasonal because of the most intensity during of vegetation and the end of the growing season. The available raw material should be processed in six months that is 13.333 kg raw material per day. Using the 10 distillers with a capacity of 300 kg per load distillation can be realized 5 – 6 loads per destillerper day if 20 workers are doing the job for 12 hours per day. Gross income is based on a product price of €1,02 / 10 ml and the annual value is about €536.118 – 1.003.029.

Keywords: *essential oil, conifer, yield, gross income, the mountain Romanija.*

Introduction

The data on the use of plants originate from the time of civilization of ancient China, India, as well as civilizations from North Africa that left written evidence about using plants to treat various diseases (Phillipson, 2001). Over time, plants have become the basis of a sophisticated traditional system of healing that continued for thousands of years, as well as today supplying humanity with new medicines based on oils derived from various tree species known as essential oils (Aryg, 2017). Essential oils are considered fragrant, easily volatile substances that come from different parts of the plant (flowers, leaves, fruits, roots, plant secretions - turpentine) and usually accumulate in glandular cells or in conducting vessels (Šikanjić *et al.,* 2005). If it is produced from green parts of coniferous and fruits, it is a clear, easily mobile liquid, colorless to light yellow in color, with a very pleasant odor whose application is aimed at making pharmaceutical and perfume-cosmetic products (Marijanović-Balaban *et al.,* 2013). Fir essential oil (*Abies*) enhances the secretion of mucous membranes and as such it is used in medicine as an expectorant, while spruce oil (*Picea*) is used to treat neuralgia and mucositis. Essential oils of white pine (*Pinus silvestris*) and spruce (*Picea*) have a bactericidal effect and accordingly find application in inhalation in lung diseases and

bronchitis (Milić-Matović, 2003). Under normal conditions, they are in a liquid state and are distilled with water vapor, which is used in the isolation of essential oil from plant material (Kapetanović, 1986). Therefore, it is most often obtained by hydrodistillation, a technique for separating substances that do not mix or mix very poorly with water and that are unstable at the boiling point (Pavičević, 2016) between 50 ° C and 320 ° C (Kapetanović, 1986). The extraction process provides high quality oil and it is a relatively simple, safe process (Pavičević, 2016) that lasts 2-3 hours (Marijanović-Balaban *et al.*, 2013) and it is in line with environmental protection. Besides the classic hydrodistillation with electric heating, the hydrodistillation improved by the introduction of microwave radiation is also applied (Pavičević, 2016). After the production process there are cooked green parts of conifer trees, which can be used for energy purposes and also used for humus production by the process of rotting. For an additional equipment and a more complex technological process, wood wastes, may also be used for the manufacture of fodder yeast, whereby a high-value protein nutrition is obtained from cellulosic material (Šikanjić, 1996).

Forests of the mountain Romanija (Entity of Republic of Srpska, Bosnia and Herzegovina) are rich in coniferous species (*Abies alba, Picea abies, Pinus silvestris, Pinus nigra*) presenting the base of growing stock with 134.000 m^3 of annual cut. After timber harvesting in forest there are side products as well needles, strobiles, offshoots and branch mid-diameter 1 – 4 cm that do not have any use, but they are actually the raw material for essential oil production. This research is aimed at exploring the possibility of essential oils rational production extracted from green parts of coniferous trees on the Romanija mountain.

Material and Methods

The study was conducted using general scientific method analysis, synthesis, induction and deduction and from special scientific methods analysis of time series through trend formulation. The average annual exponential growth rate (AEGR) was used to estimate the rate of change of the observed quantities and was calculated via the exponential regression curve (Keča, 2014). Using the survey, the data were collected from forest management unit (FMU) „Romanija" Sokolac, public enterprise of forestry „Šume Republike Srpske". The questions referred to the basic data on the FMU and quantity of wood assortiment placed on the market selected by tree species. According to the obtained data and the data based on the realized placement of the total wood mass of conifers for the period from 2005 – 2014 was examined the essential oil rational production by extraction from greens parts of coniferious (*Picea abies, Abies alba, Pinus silvestris*).

The amount of small branches with leaves that remain in the forest after cuttting trees (depending on the type of trees, age, height of trees) is 20-25% of the biomass of trees (Domak *et al.*, 2001), but for research purposes it was taken the amount about 20%. Based on the total mass of coniferous crown, which includes 19-21% of the total mass of the above – ground part of the tree, the required amount for extraction of essential oil is 57% (needles, strobiles, offshoot and branches, mid-diameter 1 – 4 cm) (Šikanjić *et al.*, 2005). The total annual weight of available raw material (kg) for the essential oil distillation was obtained on the basis of the specific weight of the raw material is 150 kg / m^3 which is according to some authors only 50% of the average specific weight (Y = 300 kg / m^3) (Marijanović-Balaban *et al.*, 2013). The essential oil yield was obtained on the basis of the arithmetic value of the coefficient of yield of coniferous essential oil (*Picea, Abies and Pinus*) at the beginning (April) and at the end of the growing season (October) and amounts to 0.3725 cm^3 of oil / 100 g (Marijanović-Balaban *et al.*, 2013). Gross income was obtained on the basis of the production price of essential oil (€1,01 / 10 ml for conifers) which was formed on the basis of

the market price of the same products on the official websites of certified manufacturers (€1,53 – 2,56 / 10 ml) (01).
The survey was conducted in order to define the essential oil rational production with the purpose of its production in FMU "Romanija" Sokolac. The research topic is FMU "Romanija" and the amount of green parts coniferiuos that has not been used after cutting trees in the period from 2005-2014.

Results and Discussion

The amount needed to obtain the essential oil was determined based on the amount of wood assortiment placed and ranges from 9.384 m^3 in 2005 to 17.556 m^3 in 2013. The lowest yield of essential oil was recorded in 2005 and amounted to 5.243 liters, while in 2013 the maximum was 9.810 liters.

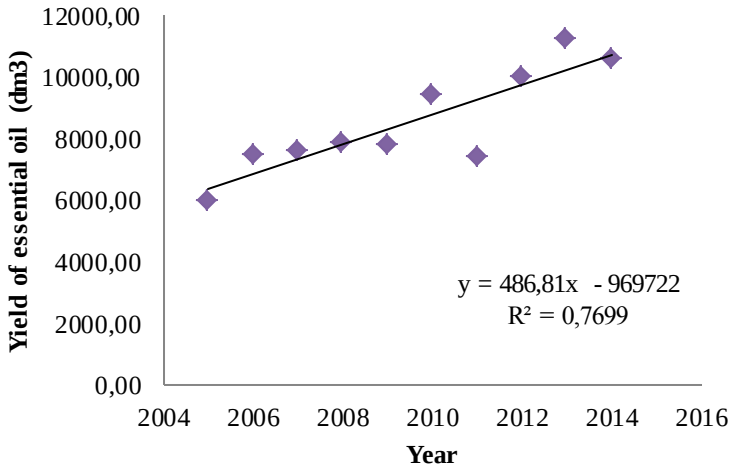


Figure 1. Yields of essential oil produces from green parts of conifer trees in the period 2005 – 2014

A very strong correlation was expressed (R = 0.769) and a correlation factor was statistically significant as indicated by an error of 0.08% (*Significance* F). The calculated parameters are significant (*P-value* or an error level of α = 0.05) (Table 1). The yield of essential oil is indirectly depending on the annual harvest of conifer species. The trend of yield of essential oil is presented by a linear trend and it has been showing a growth trend in the future with a slight decline in the 2011 (Figure 1).

Table 1. Elements of the regression analysis of the yields of esssential oil produces from green parts of conifer trees on the mountain Romanija in the period 2005 – 2014

	Параметар	t	R	F		
a	-848506.936	-5.12861	0.769897	0.084929	Y = 425,9x - 84850	
b	425.9586314	5.173687			AEGR (%)	5,7

The most significant gross revenue that would be generated by the placement of essential oil in 2013 was €1.003.029 and the lowest value was €536.118 in 2005.

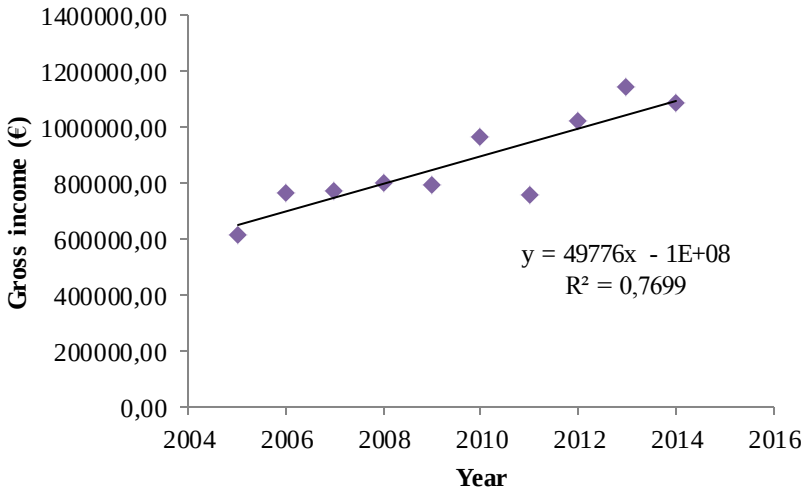


Figure 2.. Gross income of essential oil (*Abies, Picea, Pinus*) in the period 2005 - 2014

The trend of gross revenue is presented by a linear trend and it has been showing a growth trend in the future (Figure 2). Elements of the regression analysis of the gross income of esssential oil produces from green parts of conifer trees are shown in Table 2.

Table 2. Elements of the regression analysis of the gross income of esssential oil produces from green parts of conifer trees on the mountain Romanija in the period 2005 – 2014

	Параметар	t	R	F		
a	-1.7E+08	-5.12861	0.769897	0.084929	Y = 85192x – 2E+08	
b	85191.726	5.173687			AEGR (%)	5,7

The annual cutting rate of FMU "Romanija" is about 134.000 m^3, mostly coniferous species (*Picea, Abies, Pinus*) whose production is aimed at the exploitation of trees and branch timber, while brushwood and needles remain in the forest as a side product. This green part of fir, pine and spruce is the basic raw material used in the essential oil producing. Fresh felled branches (mid-diameter 1 – 2 cm on the thicker end of branch) with needles are chopped on a simple chopper mashine to a length of 3 to 5 cm for easier and faster distillation, as well as for a higher percentage of utilization (essential oil yield) (Marijanović-Balaban *et al.*, 2013). Technical, technological possibility and economic justification of the production of essential oil on semi-industrial equipment is possible if it is placed near the site from which the raw material is imported, but also provides a continuous supply of water (if the equipment works on the principle of steam distillation) (Marijanović-Balaban *et al.*, 2013). The mountain Romanija has many of natural streams (Bioštica, Kaljjina, Bereg, Vrela) which provide a constant supply of water. Gas is usually used as a source of energy for these distillates and wood can also be used (Marijanović-Balaban *et al.*, 2013). After the production process there are cooked green parts of conifer trees which can be used for energy purposes in next proceses, taking care to maintain a constant temperature during the distillation process (Marijanović-Balaban *et al.*, 2013). According to the value of annual cutting conifer trees in the forests on the mountain Romanija there are about 2.000 tones of raw material, which can be distilled and produce about 7.457 liters higt quality and market desired essential oils. The data related to the production of essential oil on small semi-industrial appliances, with a capacity of up to 100 kg of conifers, show a higher market price of the obtained oil by more than 40% compared to the production price (Marijanović-Balaban *et al.*, 2013). The production is seasonal because it is most intensive during and before the end of the growing

season. The available raw material should be processed in six months that is 13.333 kg raw material per day. Using the 10 distillers with a capacity of 300 kg per load distillation can be realized by 5 – 6 loads per destiller if working 20 worker, 12 hours per day.
By placing machines near the site where the raw material is located with a safe supply of water and maintaining a constant temperature during distillation, it would simply produce quality essential oil at low cost (Marijanović-Balaban *et al.,* 2013) and achieve significant economic benefits.

Conclusions

Based on the analysis and examination of essential oil rational production from green parts of conifer trees (*Picea, Abies, Pinus),* the following conclusions were drawn:

- the amount required to obtain essential oil ranges from 9.384 m^3 in 2005 to 17.556 m^3 (2013);
- the lowest yield of essential oil was recorded in 2005 (5.243 liters) while the maximum yield in 2013 was around 9.810 liters;
- gross income that would be realized through the sale of essential oil produced from green parts of conifer trees in the period from 2005-2015 years shows growth represented by a linear trend.

Forests on the mountain Romanija have great potential for the development of non-wood forest products (NWFPs) sector, especially in terms of essential oil production from green parts of conifer trees (*Picea, Abies, Pinus*). Having in mind the amount of raw material that remains in the forest after exploitation as a side product (needles, strobiles, offshoots and branches mid-diameter 1 – 4 cm) the yield that would be achieved by their processing is not negligible. The financial resources that would be realized through the placement of the produced essential oil range from €536.118 - 1.003.029 placed at the production price which is reduced from the market by 30-50%. Taking into account the costs required to start this production (costs of procurement of distillers and other machinery, costs of workers salaries, etc.) the profitability of such a project would be rational after the first year.
In the development of current forest management bases, NWFPs received little attention. According to the above facts, it is necessary to further explore the possibility of essential oil production from green parts of conifer trees and to organize this production by making projects for execution.

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VASCULAR FLORA IN THE FUNCTION OF RED DEER FEEDING IN MEADOWS WITHIN BOSUT FORESTS

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Abstract

Red deer occupied a lot of forest areas in the past in Serbia, but due to human activities, it gradually dissapeared. The process of reintroduction of red deer is still ongoing. For that reason, it is very significant to examine floristic composition of meadows which includes effects of many different factors such as: orography, soil, climate, vegetation. Red deer uses bark of hornbeam and beech in order to satisfy its food needs. It should be emphasised that the importance of some meadow representatives is related to the fact that they contain much more nutrients and have much higher energy value compared to the bark of above mentioned species. The research was conducted at the area of Bosut forests in order to determine abundance of herbaceous plants that are used for red deer feeding. Three localities were selected as experimental plots – one situated in dry (road between two departments) and the other two in wet (Dubovci puddle and Fish puddle) site conditions. Based on the obtained results, it can be deduced that much richer floristic diversity was recorded on wet than on dry localities – this is particularly related to species from families *Poaceae* and *Fabaceae* as the most important for red deer game feeding.

Keywords: *vascular flora, red deer, meadow communities, Bosut forests*.

Introduction

Red deer is one of the most important large game species in Republic of Serbia. As for the state of its populations, the most suitable situation is in the area of Vojvodina in municipalities of Sombor, Pančevo and Apatin (Gačić *et al.*, 2020). The most numerous and biologically and economically most valuable red deer populations in Vojvodina are located at the area of Podunavlje and Posavina, in alluvial forests of pedunculate oak, ash, hornbeam and some soft deciduous species such as willow and poplar close to the rivers Danube, Sava and Bosut, and this is suitable area for this autochthonous large game (Gačić *et al.*, 2020). The red deer is basically species of steppe grassland, due to its strong musculature and long, thin legs adapted to long running. Garnet horns of males unsuitable for movement through dense vegetation is another reason why red deer inhabits open spaces.

As for number of red deer population in Europe, it was due to excessive and uncontrolled hunting (primary cause), then unplanned deforestation, site loss and competition with domestic cattle (secondary cause) significantly decreased close to extinction in some countries such as Swiss, Slovenia, Estonia, Latvia, Lithuania or it was at the edge of extinction in Czech Republic, Portugal, Italy, Denmark, Poland (Deinet *et al.*, 2013). Using of red deer populations in Serbia is less compared to Hungary, even though the area of these countries is almost the same, and Serbia has a greater forest cover and more hunters than Hungary. On the other hand, using of this species population is even four times less than in Slovenia, even though its area is about four times less compared to Serbia and Hungary. Unlike in Serbia and

Hungary, there is also no legal obligation of game counting in the spring for hunting ground users in Slovenia (Mladenović *et al.*, 2019).

Apart from the bark of wood species, herbaceous plants are also of a big importance for red deer feeding. Among them, species belonging to families *Poaceae* and *Fabaceae* are characterized by a great content of nutrients that is connected with their high energy value. For that reason, meadows and open forest complexes with dominant role of plants from above mentioned families are a good base for red deer feeding.

Floristic composition of forests from the area of Ravni Srem is very different and rich (Gajić and Karadžić, 1991). The fact is related to a great number and cover of plants from many families. Some of them have a lot of medical properties. Some very rare species such as *Crataegus nigra*, *Nymphaea alba*, etc. are found in this area, mainly in wet localities.

The area of the researched hunting ground was 20.100 ha in 1992. Later, its overall area was reduced to 14.644 ha. At the end of 1998, the hunting ground was again reduced and divided into two hunting grounds: Bosut forests and Kućine (Ristić, 2008).

During the civil war between 1991 and 1995, red deer game was almost completely extinct from this hunting ground, while roe deer was kept to a minimum. There are a few measures that significantly contributed to the improvement of the bad conditions recorded there such as: hunting ground fencing, construction of wells and watering places, rehabilitation of hunting and technical facilities and installation of an electric fence in order to prevent damages caused by wild animals on nearby agricultural areas (Ristić, 2008).

The main scope of the paper was to establish floristic diversity and species abundance of the meadows in the area of Bosut forests with a special focus on species from the families *Fabaceae* and *Poaceae*. These plants are the most important nutritive components for red deer and its reintroduction at this area depends on them to a great extent.

Material and methods

Study area

Hunting ground „Bosutske šume" was founded at the area of forest, soil and water on the territory of municipalities Šid and Sremska Mitrovica with a whole area of 14.912 ha (Decision of Provincial Secretariat of Agriculture, Water Management and Forestry, 2012). As for position of the hunting ground, based on elevation, it belongs to plain hunting grounds. It is situated in the southern part of Vojvodina and south-western part of Srem which means it spreads in the far western part of the municipality of Sremska Mitrovica and in the southern part of the municipality of Šid. In the south, it leans on the left bank of the river Sava, while in the west it leans on the border with the Republic of Croatia. In the north it borders with the hunting ground of the Hunting Association Srem-Šid, while in the east it borders with the hunting ground Srem-Bosut. The Belgrade-Zagreb highway passes north of the researched hunting ground. From the territorial-administrative point of view, the hunting ground „Bosutske šume" belongs to the territory of the municipality of Sremska Mitrovica (Fig. 1).

Floristic research

Floristic data was collected during the vegetation season in 2020. Based on the site conditions, three representative localities (road between two departments in MU „Blata-Malovanci", Dubovci puddle in MU „Blata-Malovanci" and Fish Puddle in MU „Vratična-Cret-Carevina) were selected. Four phytocenological records (total 12) were taken in each locality. All vascular plants were recorded according to the Braun Blanquet (1964) method. Plant material was collected and transported to the Laboratory for Botany at the Faculty of Forestry - University of Belgrade. Herbarium specimens were then made and deposited in a

Great Herbarium belonging to the Chair of Seed Science, Nursery Production and Afforestation, Department for Forest Botany.
Plants determination was performed according to Gajić (1989) and Kojić (1990). Collected plants were classified into three groups: family *Poacea*e, family *Fabaceae* and another species.–Based on the obtained results we can deduce about presence and abundance of herbaceous plants within the above mentioned categories, not only in the three localities selected, but also at the whole researched area.

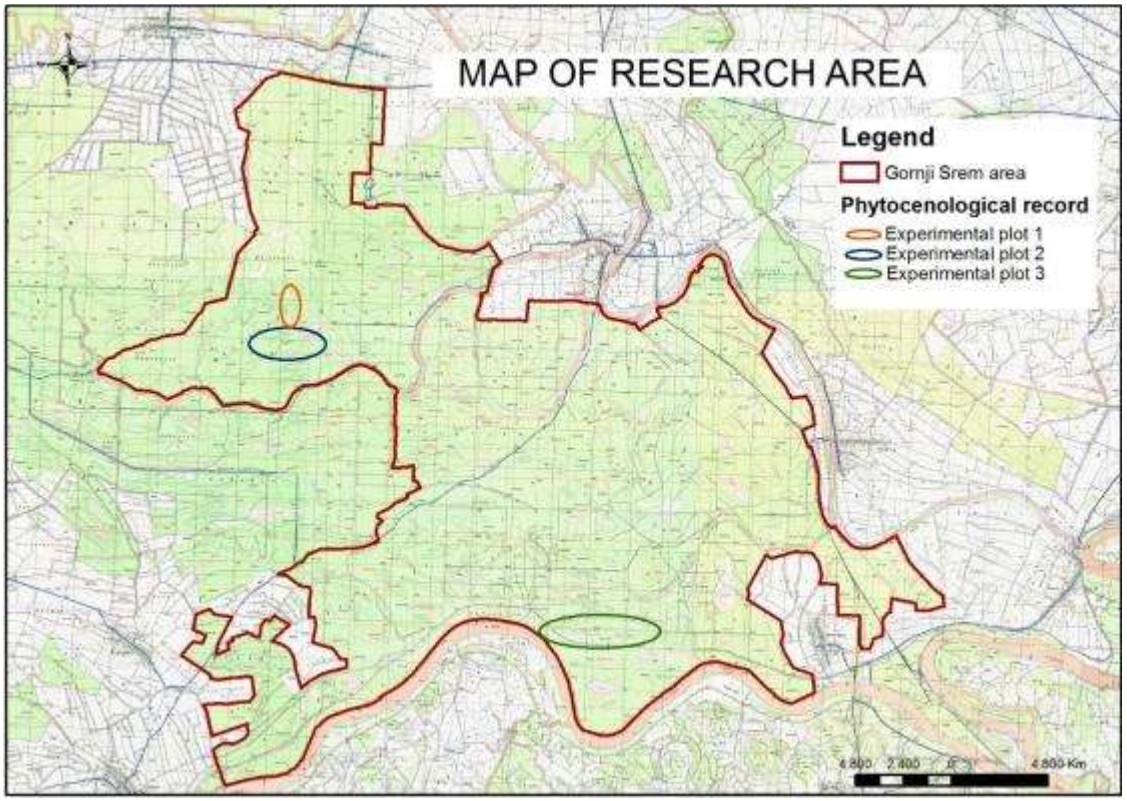


Figure 1: Map of research area with phytocenological records

Results and discussion

Locality 1 is situated in dry site and it is actually a road between departments 21 and 30 within MU „Blata-Malovanci" at the area of FA „Morović". At this locality total number of species varies between 26 and 34 (Table 1).
There are some representatives from family *Poaceae* of a high quality such as: *Agrostis alba*, *Poa trivialis*, *Lolium perenne*, *Dactylis glomerata* and *Festuca pratensis*. There are also some taxons from this family characterized by medium quality such as *Agropyrum repens* and *Bromus inermis*. Another species from *Poaceae* family have no value (Kojić, 1990). Among species from *Poaceae* family, based on measured parameters (number and cover), the most dominant are *Festuca pratensis* and *Dactylis glomerata*. As for recorded species from *Fabaceae* family in this locality, there are a few of a high quality such as: *Medicago sativa*, *Trifolium repens*, *Trifolium pratense* and *Vicia sativa*. There are just two legumes of a medium quality in this locality such as *Trifolium alpestre* and *Vicia cracca*. All other species from family *Fabaceae* in locality 1 have no value (Kojić, 1990). As for the number and cover, among all recorded species from family *Fabaceae* in this locality, the most dominant are *Medicago sativa* and *Onosis spinosa*. There are also a lot of plants with medical properties among another individuals (*Urtica dioica*, *Brassica nigra*, *Viola alba*, *Potentila erecta*, etc.).

Table 1. Number and cover of plants in „Road between departments 21 and 30" locality, MU „Blata-Malovanci"

Species	I	II	III	IV
Agropyrum repens	+	+	1.1	
Agrostis alba		+	+	+
Bromus inermis	+	+	+	
Bromus molis	+	+	+	
Bromus racemosus	+			
Dactylis glomerata	+	+	+	1.2
Festuca pratensis	1.2		1.2	+
Hordeum murinum		+	+	+
Lolium perene	+		+	+
Poa trivialis	+	+	+	+
Sorghum halepensis	+	+	+	
Σ (**Poaceae**)	**9**	**8**	**10**	**6**
Medicago sativa	2.2	2.1	+	+
Onosis spinosa	2.1	+	+	
Trifolium alpestre	+			+
Trifolium pratense	1.1	1.1	+	+
Trifolium repens	+	+	1.1	1.1
Vicia cracca	+	+	+	+
Vicia dumetorum	+	+	+	
Vicia sativa	+		+	+
Σ (**Fabaceae**)	**8**	**6**	**7**	**6**
Σ (**Other species**)	**13**	**20**	**17**	**14**
Σ (**Total number**)	**30**	**34**	**34**	**26**

In the second locality, situated at the area of Dubovci puddle within MU „Blata-Malovanci", FA „Morović", the total number of recorded species was between 36 and 44 (Table 2).
As for the plants from the family *Poaceae*, some of them are of a high quality such as: *Agrostis alba*, *Dactylis glomerata*, *Festuca pratensis*, *Lolium perenne*, *Poa pratensis* and *Poa trivialis*. Some species of a medium quality such as: *Agropyrum repens*, *Bromus inermis* and *Cynodon dactylon* were also recorded. All other taxons from *Poaceae* family at this locality have no value (Kojić, 1990). Based on values of the measured parameters (number and cover) for individuals from *Poaceae* family, the most dominant are - *Bromus inermis, Bromus mollis* and *Agroyprum repens*. As for the plants from *Fabaceae* family in this locality, there are a few of a high quality such as: *Lotus corniculatus*, *Medicago lupulina*, *Medicago sativa*, *Trifolium repens*, *Trifolium pratense*, *Vicia sativa* and *Vicia pannonica*. There are two species from the legumes that are of a medium quality such as *Trifolium alpestre* and *Vicia cracca*. All other plants from *Fabaceae* family in this locality have no value (Kojić, 1990). Among the legumes, *Trifolium pratense* and *Trifolium repens* are the most dominant based on the number and cover. There are also a lot of species with medical properties recorded in this locality (*Achillea milefolium*, *Althaea officinalis*, *Arctium lappa*, *Symphytum officinale*, etc).

Table 2. Number and cover of plants in „Dubovci puddle" locality, MU „Blata-Malovanci"

Species	I	II	III	IV
Agropyrum repens	+	+	1.2	
Agrostis alba	+	+	+	+
Bromus inermis	2.2	+		+

Bromus commutatus	+	+	+	
Bromus molis	+	+	1.2	1.1
Bromus racemosus	1.2	+	+	1.1
Bromus sterilis	+	+	+	
Calamagrostis epigeiois		+	+	+
Cynodon dactylon	+	+	+	+
Dactylis glomerata	+	+	+	+
Deschampsia cespitosa	+		+	
Festuca pratensis		+	+	+
Hordeum murinum	1.1	1.1	1.2	1.1
Lolium perene	+	+	1.1	1.1
Poa pratensis	+	+		+
Poa trivialis	+		+	+
Sorghum halepensis	+	+		+
Σ (**Poaceae**)	**15**	**15**	**14**	**13**
Coronilla varia	+	+	+	+
Lathyrus tuberosus	+	+		+
Lotus corniculatus	+		+	
Medicago lupulina	+	+	+	
Medicago sativa	1.1	+	+	+
Onosis spinosa	+	+		+
Trifolium alpestre	+		+	
Trifolium pratense	+	+	1.2	1.2
Trifolium repens	+	+	1.2	1.2
Vicia cracca	+	+	+	+
Vicia dumetorum	+	+	+	+
Vicia pannonica	+	+	+	+
Vicia sativa	+	+	+	+
Σ (**Fabaceae**)	**13**	**11**	**11**	**10**
Σ (**Other species**)	**16**	**17**	**15**	**13**
Σ (**Total number**)	**44**	**43**	**40**	**36**

In the third locality, situated at the area of Fish puddle within MU „Vratična-Cret-Carevina", FA „Višnjićevo", total number of recorded species varied from 38 to 46 (Table 3).
There are a few species from *Poaceae* family of a high quality recorded in this locality such as: *Agrostis alba*, *Dactylis glomerata*, *Festuca pratensis*, *Lolium perenne*, *Poa pratensis* and *Poa trivialis*. There are also two taxons of a medium quality - *Bromus inermis* and *Poa annua*. The other species in this locality have no value (Kojić, 1990). Among the recorded plants from *Poaceae* family, based on the measured number and cover, the most dominant are *Dacytlis glomerata* and *Hordeum murinum*. As for the plants from *Fabaceae* family, there are a few of high quality in this locality such as: *Lotus corniculatus*, *Medicago lupulina*, *Medicago sativa*, *Trifolium repens*, *Trifolium pratense*, *Vicia sativa* and *Vicia pannonica*. There were also recorded two legumes of a medium quality such as *Trifolium alpestre* and *Vicia cracca*. All other legumes in this locality have no value (Kojić, 1990). The most dominant plants from *Fabaceae* family in this locality are: *Trifolium repens*, *Trifolium pratense* and *Coronilla varia*. This locality is also characterized by the presence of a large

number of medicinal plants (*Achillea milefolium*, *Althaea officinalis*, *Arctium lappa*, *Symphytum officinale*, etc).

It should be emphasised that natural meadows and grasslands occupy a lot of space in the Republic of Serbia and they are actually ecosystems made of herbaceous communities with high coverage. These associations mainly consist of perennial herbaceous mesophytes. There have been a wide range of species in communities like these, from very harmful, even toxic, to some very valuable (Gačić *et al.*, 2008; Tomić *et al.*, 2010).

If we compare the obtained results about the floristic composition in hunting grounds in the area of mountain Tara (Gačić *et al.*, 2020) to the results in this paper, we can deduce that a number of recorded species within phytocenological records and a range of variation differ a little from each other. However, there are more species of a high quality from *Fabaceae* family, and more taxons of a high and medium quality from *Poaceae* family, as well, at the area of Bosut forests, before all at wet sites compared to hunting ground on mountain Tara.

Table 3. Number and cover of plants in „Fish puddle" locality, MU „Vratična-Cret-Carevina"

Species	I	II	III	IV
Agropyrum repens	+	+	+	+
Agrostis alba	+		+	+
Bromus inermis	+	+		+
Bromus molis	+	+	+	+
Dactylis glomerata	2.1	2.1	+	1.2
Festuca pratensis	+		+	
Hordeum murinum	2.1	+	+	2.1
Lolium perene	+		+	
Poa annua	+	+	+	
Poa pratensis	+		+	+
Poa trivialis	+	+	+	+
Sorghum halepensis	+		+	
Triticum villosum	+	+		+
Σ (**Poaceae**)	**13**	**8**	**11**	**9**
Coronilla varia	+	1.2	+	1.2
Lathyrus tuberosus	+	+	+	+
Lotus corniculatus	+		+	+
Medicago lupulina	+	+	+	
Medicago sativa	+	+	+	+
Onosis spinosa	+		+	
Trifolium alpestre	+	+		+
Trifolium pratense	+	+	1.2	1.2
Trifolium repens	+	+	1.2	1.2
Vicia cracca	+	+	+	+
Vicia dumetorum	+	+	+	
Vicia pannonica	+		+	+
Vicia sativa	+	+	+	+
Σ (**Fabaceae**)	**13**	**10**	**12**	**10**
Σ (**Other species**)	**20**	**22**	**22**	**19**
Σ (**Total number**)	**46**	**40**	**45**	**38**

Conclusions

Research was conducted in meadows at the area of Bosut forests. The main scope was to determine the presence and abundance of the species from *Poaceae* and *Fabaceae* families as the main nutritive elements for red deer. Bearing in mind that reintroduction of this autochthonous large game on the territory of Vojvodina is still ongoing, it is necessary to examine nutritive potential of meadows and grasslands in this area. Much richer floristic diversity was recorded in the wet than in the dry site conditions. It should also be emphasised there was found a great number of medicinal plants at the whole research area. One of the important recommendations related to the sustainable management of red deer game under reintroduction conditions refers to achieving balance between the number of their populations and the adequate feeding conditions. Due to that, one of the measures should be increasing of the areas covered with species of a high value from *Poaceae* and *Fabaceae* family by sowing the existing meadows.

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EFFECT OF STRATIFICATION ON SEEDS GERMINATION AND SEEDLING GROWTH OF *FAGUS SYLVATICA* L.

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Abstract

The European beech (*Fagus sylvatica* L.) is one of the most important broadleaved species in European forestry, which grows in the Republic of Moldova on approximately 1,450 hectares of natural protected areas. Beech is propagated by seeds, but its seed formation is irregular. Therefore it is necessary to store the seeds and to obtain a high germination percentage, which could be maximise by cold stratification. The aim of our research was to study the germination of *Fagus sylvatica* L. seeds after cold stratification by followed characteristics: germination energy; total germination; coefficient of speed germination; mean daily germination; mean germination time. The beech seeds were exposed to stratification at a temperature of +4±1°C and a humidity of 30%. The seeds germination started on tenth day and continued during three months. The maximum of germination energy (calculated on 30th day) and total germination was equal to 27.0 and 72.3%, respectively. Mean germination time was 55.48±5.72 days, and coefficient of speed germination constitued 1.72±0.24. The germinated seeds were sown and the bio-morphological features of beech seedlings were studied in nursery condition. The period of seedling appearance after sowing of germinated seeds was minimum 10 days, maximum 29 days. The proportion of grown seedlings from germinated seeds was about 69.2%. A clear dependence of seedling number on stratification time of beech seeds was not established. The chlorophyll index of seedling leaves after 45 days of growth was determined and compared with a similar leaf index in adult plants of European beech.

Keywords: *Fagus sylvatica* L., *seed, stratification, germination, seedling growth.*

Introduction

Fagus sylvatica L. (European beech) is common for forests of different European countries, from southern Sweden to central Spain.The natural range of the European beech are limited by climatic conditions. The arid climate and reduction less than five months of growing season inhibits the spread of this species. Moreover, the plant does not survive in winter by prolonged decrease in the average temperature below –5°C, with some exception, European beech can resist short-term frosts of –25ºC – 35°C (Bonner and Leak, 2008; Gömöry *et al.*, 2010; Sułkowska *et al.*, 2012). European beech is a highly competitive species and is relatively resistant to periods of drought (Roloff and Grundmann, 2008). However, period more than 2 months (10 weeks and more) of severe drought can adversely affect plant survival (Geßler *et al.*, 2007; Milad *et al.*, 2011; Pflug *et al.*, 2018).

The most eastern border of the species range *F. sylvatica* passes through the territory of the Republic of Moldova. Protected beech forests occupy an area of about 1,450 hectares and are available in nine conservation areas of the republic, including in two scientific reserves "Plaiul Fagului" and "Codrii" (Postolache and Postolache, 2010; Postolache and Lozan, 2011). However, the state of beech forests in the Republic of Moldova is a matter of concern and requires urgent action. The occurring global climate changes, as well as extreme weather events such as icing, droughts, and wet-snow precipitation in the spring after sap flow and leaf

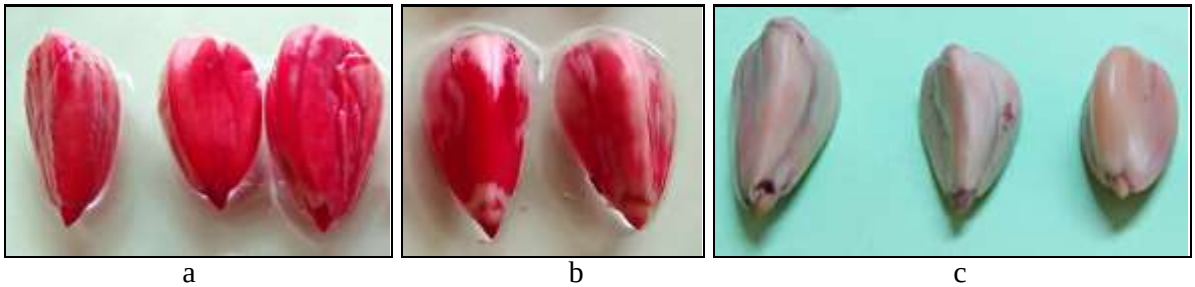
development significantly affect forest ecosystems. In the Republic of Moldova, over the past few decades, tendencies to increase the maximum air temperature have intensified, as well as an annual precipitation is also below the norm, especially in the spring and summer. Therefore, in this region, where *F. sylvatica* is actually growing near its drought limit, the species especially needs to be studied for protection and to maintain its population at the proper level.

It is well known that European beech trees begin to fruit formation from about 20-80 years of age, depending on the growing conditions –alone or in stands. According to Bonner and Leak, (2008), large beech seedcrops occurs no more often than once every three years and even once every 15-20 years, in dependence on the location of growth, climatic and other conditions. The most crucial issues related to the reproduction of beech forests also rest against the problem of storage after collection of seeds and seedling growth after germination. Beech seeds are characterized by strong exogenous and deep physiological dormancy; they are referred to long-growing seeds that need a long period of stratification. At the same time, seed germination during stratification is greatly extended, therefore, resorting to various methods to accelerate this process (Pawłowski, 2007; Procházková and Bezděčková, 2008; Bezděčková *et al.*, 2013). The aim of our research was to study the germination rate of *F. sylvatica* seeds after cold stratification in humid conditions and seedling emergence after sowing of germinated seeds.

Materials and Methods

The experiments were carried out in the Laboratory of Natural Bioregulators of the Institute of Genetics, Physiology and Plant Protection, Republic of Moldova during 2019-2020. Due to the fact that no fertile beech seeds were found on the territory of the Republic of Moldova in the season of 2019, the seeds of European beech, *Fagus sylvatica* L. (Fagaceae) were kindly provided to us by our colleagues from the Slovak Republic (seeds were collected in Tribeč Mountains, Western Carpathians). Initially, the beech seeds were dried at ambient temperature and humidity until they reached a moisture content of 8-10% (fresh weight basis). Then they were stored at the temperature of $+4\pm1^{o}C$ in plastic bags, and placed in plastic containers.

The viability of seeds was determined by tetrazolium test (Kerkez *et al.*, 2018) using for seed staining by the 2.3.5-triphenyltetrazolium chloride (2.3.5-TTC) solution. In dependence of the seed staining, the seeds were distributed into three groups: a) the seeds with bright red staining, which are completely viable and give normal seedlings; b) partially stained seeds that may produce either normal or abnormal seedling; c) greyish stained or black seeds, indicate the presence of a dead tissue in the seed, they are non-viable (Fig. 1). If the test performed appropriately, the percentage of viable seeds obtained by tetrazolium assay is very close to the percentage of seed germination expected under most favourable conditions (Verna and Majee, 2013). The average seed assessment by groups was used as an input for the correlation analysis with seed germination data.



a b c

Figure 1. Determination of the viability of *Fagus sylvatica* seeds depending on the degree of staining using the tetrazolium test (a – the seeds with bright red staining; b – partially stained seeds; c – greyish stained or black seeds).

Germination evaluation. The dormancy of seed was broken as following: seeds were kept in water until moisture content reached to 30% (fresh weight basis) and were subjected to cold stratification at +4±1°C for up to 95 days. The germination test (four replicates of 50 seeds each) was carried out in accordance with the recommendations of the International Seed Testing Association (ISTA, 2006).

Germination was defined as the first emergence of cotyledons (Fig. 2a). The daily germination counts were made on the seeds until no further germination occurred. The following observations: total germination percentage (TGP), mean daily germination (MDG), mean germination time (MDT), germination rate index (GRI) were made (Al-Ansari and Ksiksi, 2016).



a b

Figure 2. Determination of the germination of *Fagus sylvatica* seeds during stratification (a – radicle breaking through the nut wall; b – germinated seeds).

Seedling emergence studies were carried out in laboratory conditions (constant temperature 18–20°C) and in a greenhouse (temperature depended on weather, the fluctuation of temperature in first month of seedlings growth was from 21°C to 30°C and in second month – from 22°C to 38°C). Peat with pH = 5.5 was used as a substrate. Seeds were sown as they were germinated (Fig. 2b). Daily monitoring of seedling growth and development of plants was carried out.

Seedling emergence was assessed after the appearance of hypocotyl or cotyledons left the soil (Fig. 3). Further, the assessment of the total proportion of emergent seedlings and development of plants continued until the appearance of the first and second pairs of juvenile leaves. Data are presented as the means and standard deviation of four biological replicates. The relationship between particular parameters was examined using Pearson's correlation coefficient analysis.



a b c d

Figure 3. Seedling growth stages of *Fagus sylvatica* (a – hypocotyl emerging from soil; b, c, d – cotyledons left the soil).

Relative chlorophyll content was quantified as the chlorophyll content index (CCI), measured with the Chlorophyll Meter (USA). For both, the adult tree and young seedling leaves were measured CCI. The measurements were carried out on young plants, for the first and second pair of juvenile leaves, and for adult plants, on new leaves of the current year, of the same age as the leaves of young plants. For each leaf, ten measurements on different locations were taken, avoiding the mid-vein (Van Wittenberghe, 2012).

Results and Discussion

The weight of beech seeds with moisture content of 8-10% was measured. The average weight of 100 beech seeds was 29.17±0.33g. The data obtained are similar with the data of other authors. According to various works, the weight of 1,000 beech seeds (with a moisture content of 8-10%) ranges from 100 to 350 g, and the average weight of one seed is 0.215-0.250g (Bonner and Leak, 2008; Drvodelic *et al.*, 2011).

Seed viability. The proportion of seeds with visible damages caused by insects was 8% of all collected. The seeds with mechanical injury amounted to about 4%. Through application of tetrazolium test, it was determined that only 36.0±3.7% of beech seeds were absolutely viable. The total number of viable and conditionally viable seeds (according to the methodology – partially stained seeds) reached to 69.5% Thus, in this batch of European beech seeds the proportion of non-viable seeds were equal to 30.5%. According to other authors, seed viability can vary from about 34.8-39.3% (Drvodelic *et al.*, 2011) to 66-85% (Procházková and Bezděčková, 2008; Novotný and Frýdl, 2010) and depends on many factors, on the particular batch of seeds as well as on the conditions and duration of storage.

Germination test. As a result of observations, the first germinated seeds were identified on the 10th day of the cold stratification. The germination characteristics of European beech seeds calculated after 95 days of stratification are presented in Table 1. It is necessary to mention that results of seeds germination during stratification and viability of seeds determined by the tetrazolium method were positively correlated (coefficient of Pearson correlation r = 0.989) (Table 1).

Table 1. Germination characteristics of European beech seeds

Repetition	Total number of viable seeds and conditionally viable (tetrazolium method)	Total germination(%)	Mean daily germination	Mean germination time (days)	Germination rate index
1	72,00	70.19	0.74	52.61	1.95
2	54,00	64.22	0.68	53.88	1.42
3	76,00	72.28	0.76	63.94	1.64
4	76,00	71.00	0.75	51.48	1.87
Average	69.50±10.50	69.42±3.57	0.73±0.04	55.48±5.72	1.72±0.24
Pearson coefficient	r = 0.989				

Seedling emergence. The first seedlings in the laboratory conditions were observed on the 10th day after sowing of pregerminated seeds, while in the greenhouse on the 7th day. The maximum rate of emergent seedlings reached to 50.6 and 32.8%, respectively, in the laboratory and in the greenhouse (Fig. 4).

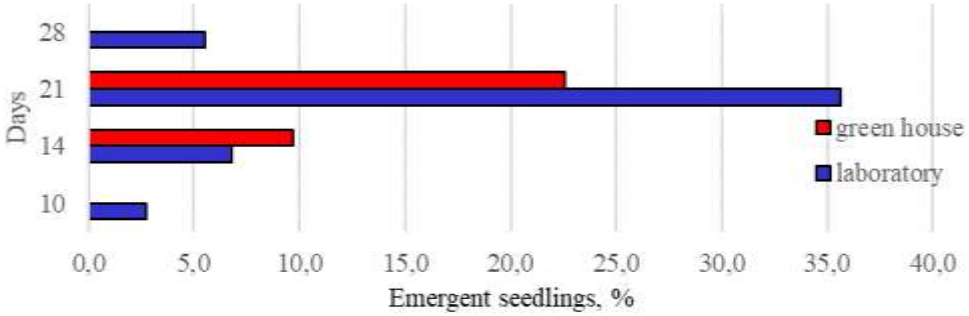


Figure 4. Total proportion of emergent seedlings of *Fagus sylvatica* in the laboratory and greenhouse conditions (% of sown seeds)

Thus, part of the seeds germinated during stratification did not survive after sowing. The critical period for seedlings emergence is the period from seed germination to seedlings rooting. The stresses of rapid temperature modification in greenhouse and lack of moisture adversely affected the survival of seedlings; when the top soil layer dries to a depth of 5 to 10 cm, 11.5 to 90.9% of beech seedlings died. The reduction in the period of seedlings emergence in the greenhouse is associated with a higher air temperature (by 4-10^{o}C) during the day compared to the laboratory. At the same time, lower and constant daily average temperatures in the laboratory conditions contributed to a higher percentage of surviving seedlings. One interesting detail should be noted – in the laboratory conditions all seedlings did not branch and had one stem during the monitoring (until the 4th pair of juvenile leaves). In a greenhouse, 5.6% of the plants branch into two or three stems immediately after the appearance of first pair of juvenile leaves.

Relative chlorophyll index was 146.0±12.5 and 154.7±7.7 g/m^2, respectively for seedlings leaves and adult plant leaves. It was found that adult beech plants compared to seedlings have a higher concentration of chlorophyll in the leaves. It was established that the chlorophyll index in the leaves increases with rising age of the seedlings (Table 2).

Table 2. Chlorophyll index of seedlings leaves

Date of measurement	The average value of the chlorophyll index of seedlings leaves, depending on the date of seed sowing			
	10.03.20	13.03.20	16.03.20	23.03.20
09.06.20	164.5	142.0	139.8	137.6
23.06.20	190.0	156.0	150.0	148.7

Conclusions

The total germination of European beech seeds as a result of stratification at a humidity of 30% and a temperature of +4±1^{o}C, was 69.42±3.57, which positively correlated (r = 0.989) with the percentage of viable seeds determined using the tetrazolium test. The maximum rate of seedlings emergence (50.6%) after seeds sowing was higher in laboratory conditions compared with the greenhouse (32.8%). The temperature and water regimes also significantly affect the emergence of the sown seeds. At temperatures above 30±2^{o}C, the number of

emergent seedlings was significantly reduced, and moisture deficiency also caused death of seedling because of underdeveloped root.

Acknowledgements

The research was carried out in frame of State programs of the Republic of Moldova by financial support of Project 20.80009.7007.07. The authors gratefully acknowledge Jan BRINDZA, doctor-ing., from Slovak University of Agriculture in Nitra, who help us in obtaining beech seeds in the Slovak Republic.

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RESULTS OF MULTIANNUAL MONITORING OF TREE CROWN CONDITION ON ICP LEVEL II PLOTS IN AUTONOMOUS PROVINCE OF VOJVODINA (SERBIA)

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Abstract

Forests are threatened by numerous biotic and abiotic damaging factors. Continuous monitoring of forest conditions is of great importance for detecting changes in forest ecosystems and determining the cause and relationships between the resulting changes and factors that affect forests. The first symptom of forest dieback is the appearance of defoliation of trees. This paper presents the results of several years of monitoring of trees defoliation of sessile oak and pedunculate oak, on two ICP level II plots. First plot was in sessile oak forest on Mt Fruška Gora in Serbia and second plot was at pedunculate oak stand near town Odžaci. Data were collected according to ICP Forests protocols from 2010 to 2019. In addition, impact of biotic and abiotic factors harmful to the condition of the trees was evaluated and analyzed. Significant level of tree defoliation was found in trees of sessile oak and pedunculate oak. At pedunculate oak we noted a low percentage of trees without significant defoliation. During the 10-year period, 7.5% of sessile oak trees and 12.5% of pedunculate oak trees died out. Investigation showed a very strong insect defoliators and oak lace bug attack influence on the results of defoliation assessments of sessile oak and pedunculate oak trees.

Keywords: *monitoring, crown condition, defoliation, insects*

Introduction

Forests as a natural resource are of great economic and environmental importance. Today, forest ecosystems are endangered by a large number of harmful factors that can be divided into abiotic, biotic and anthropogenic. Continuous monitoring of forest is of great importance for the timely detection of changes in forest conditions and for the detection of causal relationships between harmful factors that threaten forest ecosystems and forest conditions. In Europe, there was an intensive decline in forest ecosystems in the 80s of the 20th century. Air pollution was then identified as the primary cause of this decline. The International Co-operative Program on the Assessment and Monitoring of Air Pollution Effects on Forests was established in December 1984 (Nevenić et al., 2005). The program is implemented today in 42 European countries on about 6,000 Level I plots and about 500 plots with Level II monitoring.

Today, the purpose of this international program is to monitor the impact of air pollution on forest ecosystems, but also to monitor changes in forest conditions related to the effects of other harmful factors. The parameters used for continuous monitoring of forest health are the intensity of defoliation and the intensity of damage on trees caused by the action of a large number of different harmful factors. The paper presents the results of ten- year monitoring of the condition of sessile oak and pedunculate oak trees on two plots of the Level II of monitoring according to the methodology prescribed by the International Co-operative Program on the Assessment and Monitoring of Air Pollution Effects on Forests.

Material and methods

The condition of sessile oak (*Quercus petrea* L.) trees on the plot on Fruška gora (N 45º 09′ 26″, E 19º 48′ 39″) and pedunculate oak (*Quercus robur* L.) on the plot in the forest Branjevina near Odžaci (N 45º 27′ 17″, E 19º 10′ 28″) was monitored. These plots belong to the Level II plots system within the monitoring of the impact of air pollution on forests (ICP Forests).

Table 1. The description of stands where the monitoring was performed

Locality	Species	Association	Soil type	Altitude (m)	Forest stand type
Fruška gora	*Sessile oak*	*Quercetum montanum typicum*	*acid brown forest soil*	480 - 495	*coppice stand*
Branjevina	*Pedunculate oak*	*Aceri tatarico - Quercion*	*humoglay*	81 - 82	*high stand*

The monitoring was performed on the 50 x 50-meter plots. All rating trees were numbered. Dominant, codominant and subdominant trees were selected for evaluation. The assessment of the condition of the tree crowns was performed every year during July in the period from 2010 to 2019. The assessment was performed according to the methodology prescribed by the International Co-operative Program on the Assessment and Monitoring of Air Pollution Effects on Forests (Anonymus, 2010; Eichhorn et al., 2016). After evaluation, the trees were classified into the classes which are listed in Table 2.

Table 2. Defoliation clasess

Defoliation clasess	Percentage of leaf loss
0 Not defoliated	0 – 10%
1 Slightly defoliated	>10 – 25%
2 Moderately defoliated	>25 – 60%
3 Severely defoliated	>60 – <100%
4 Dead	100%

Besides, the influence of biotic and abiotic harmful factors on the condition of tree crowns was monitored in the form of an assessment of the intensity of damage due to the action of a harmful factor. Damage from a certain harmful factor was assessed on a scale with an interval of 5% (0 = not damaged tree; 5; 10; 15 ... 100 = dead tree).

Results and discussion

Ten-years period assessment of the defoliation classes and the impact of harmful factors on trees can be used to make some conclusions about trends in oak forests. In the period from 2010 to 2019, the percentage of sessile oak trees without defoliation was from 0 to 52.5%. In the period 2010-2012, the dominant share of slightly and moderately defoliated trees was found to be a consequence of attack by defoliator insects in these years (Fig. 1). Moderately defoliated trees were especially present in 2010 with 78% of the total number of assessed trees, and then the strongest attack of early defoliator insects was determined (Fig. 2). It is very significant that after 2013, the share of trees with defoliation above 25% decreased,

which is considered as a good indicator of the vitality of forest ecosystems (Potočić et al., 2008).

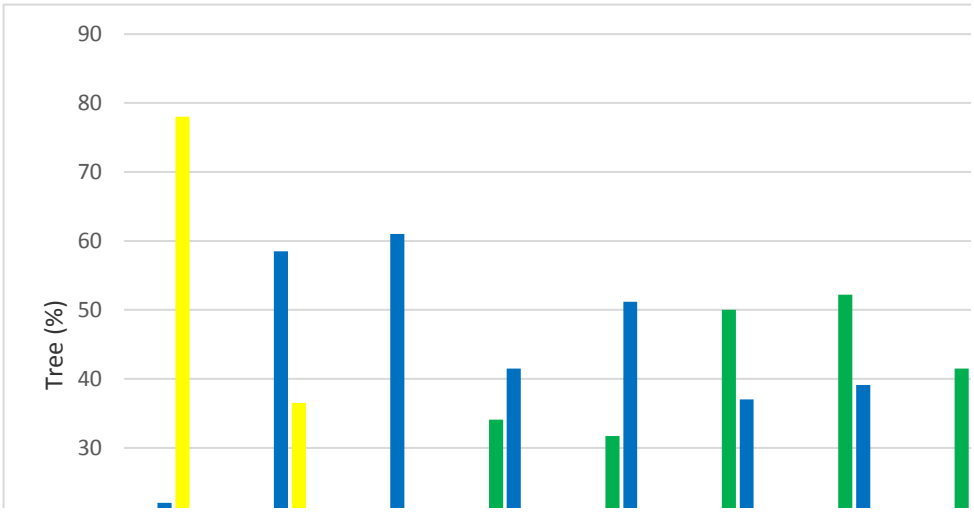


Figure 1. The distribution of *Q. petrea* trees by classes of defoliation in the period 2010-2019.

The average defoliation of sessile oak trees caused by early defoliators in 2010 and 2011 was 25.95% and 21.5%, respectively. After that, it significantly decreased and since 2015 no defoliator attack has been recorded (Fig. 2).

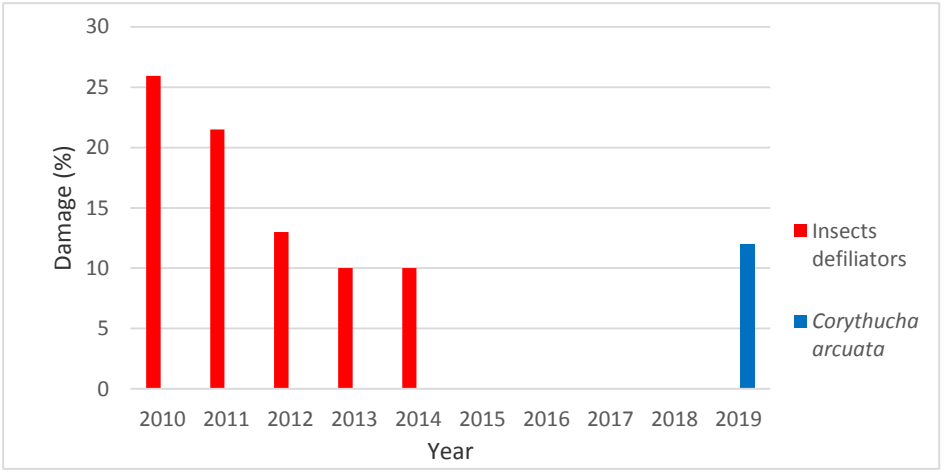


Figure 2. Average damage level of *Quercus petrea* trees in the period 2010-2019.

Of the insects from the group of early defoliators, winter moth (*Operophtera brumata* L.) and oak leaf rollers (*Tortrix* sp.) were predominantly present. Damage from oak lace bug (*Corythucha arcuata* Say) was found on the largest part of the crowns of the assessed sessile oak trees in 2019, which resulted in a smaller decrease in the share of trees without significant defoliation. In the period from 2012 to 2014, the occurrence of drying was recorded in a total of 7.4% of the assessed sessile oak trees. The drying of sessile oak trees occurred due to physiological weakening caused by drought and insect attack.

In the period from 2010 to 2019, a relatively low number of trees without defoliation (2% - 29.1%) was found for pedunculate oak, which is an even more unfavorable situation compared to sessile oak trees. In almost all years of monitoring, pedunculate oak was characterized by slight defoliation for the largest number of trees, while the percentage of trees without defoliation and with moderate defoliation varied significantly (Fig. 3). The percentage of trees with defoliation above 25% that most reliably speaks of forest vitality was

between 25% and 60.4% in 2011 and 2018, respectively. As a consequence of insects' early defoliators attack and drought, the number of trees with slight and medium defoliation was high in the period from 2010 to 2012.

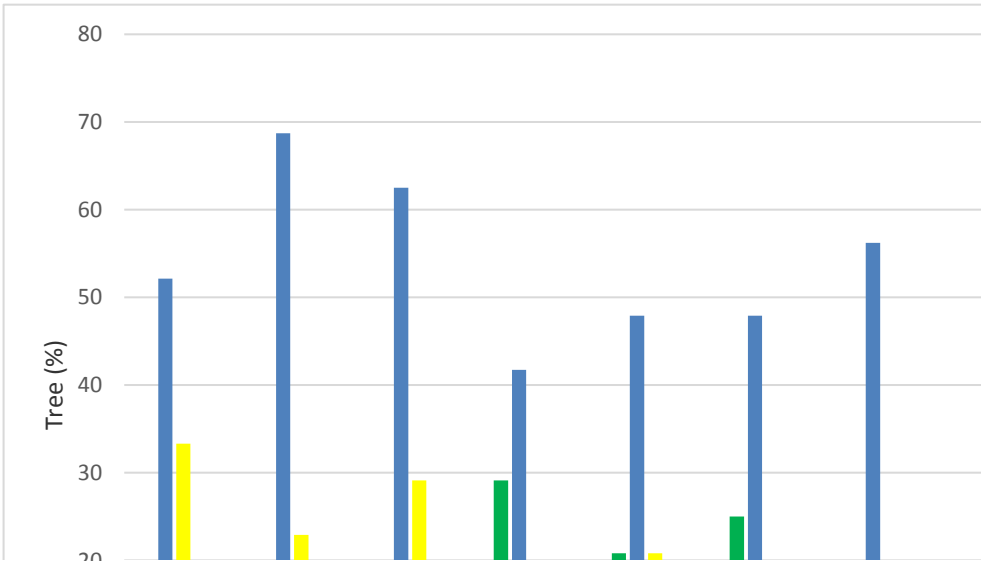


Figure 3. The distribution of *Q. robur* trees by classes of defoliation in the period 2010-2019.

Defoliation caused by insects was in the period 2011-2013 estimated at an average of about 10% of the leaf area of the assessed trees (Fig. 4). Damage from winter moth caterpillars (*Operophtera brumata* L.) was dominant. As a consequence of the occurrence of the oak lace bug (*C. arcuata*) and its harmful impact since 2017, the percentage of pedunculate oak trees without defoliation fell to below 10% of the total number of assessed trees.

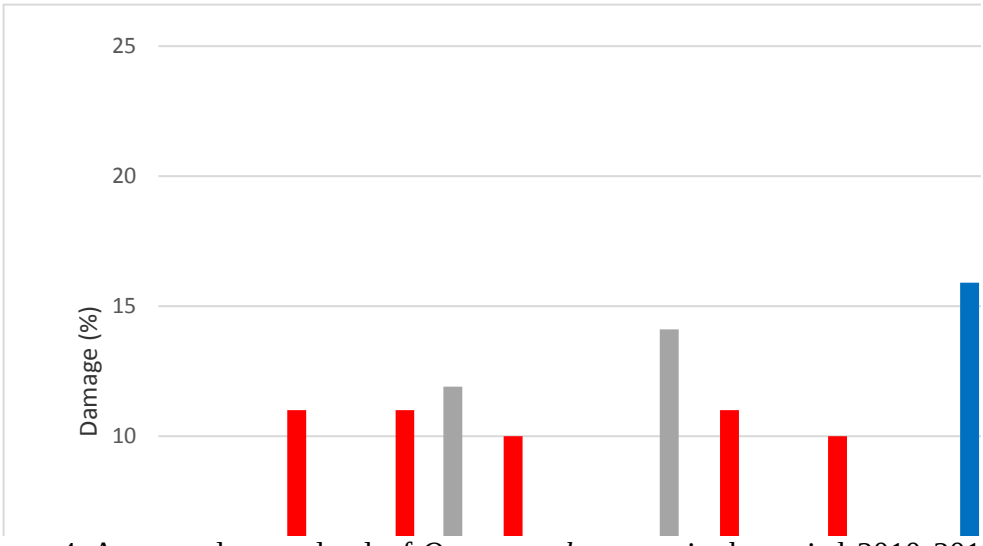


Figure 4. Average damage level of *Quercus robur* trees in the period 2010-2019.

From 2010 to 2019, drying of 12.5% of the assessed pedunculate oak trees was recorded. In the observed period, two separate waves of pedunculate oak trees drying were recorded, the first after the dry years 2011 and 2012, and the second was also a consequence of the drought in 2017 and the attack of oak lace bug which negatively affects pedunculate oak (Nikolić et al. 2019). The results of monitoring of the tree crowns condition on two plots of the second level indicated great influence of insects on the defoliation of trees. A similar conclusion was reached by Jactel & Vodde (2006) who, analyzing ICP Forests data from 21 European countries for the period from 1994 to 2005, concluded that biotic harmful factors cause 60%

of damage, and abiotic and anthropogenic 20% each, as well as that the damage occurs much more often on deciduous species. Insects and pathogens are thought to potentially play a very significant role in forest degradation under the influence of climate change (Bussotti & Pollastrini, 2017). Drought in 2011, 2012 and 2017 had a negative impact on the condition of the evaluated sessile and pedunculate oak trees. Pap et al. (2017) state that in Vojvodina from November 2016 to March 2017 a deficit of precipitation of 39.0% was recorded, and in the vegetation period of 2017 a deficit of 20.4% in relation to the multi-year average was observed. In the years immediately after the drought, an intensive occurrence of tree drying was recorded, which coincides with the statements of Dobbertin (2005), that the drought does not show its negative effect in the year when it is pronounced but in the following years.
The analysis of the results of monitoring the tree crowns condition on Level I plots and monitoring the impact of air pollution on forests in Vojvodina also showed a high degree of crown damage and widespread drying for pedunculate oak (Drekić et al., 2013; 2016). The phenomenon of pedunculate oak drying in Serbia is a problem that has been present for a long time (Grbić et al., 1991; Medarević et al., 2009), while the problem of drying of sessile oak in Serbia was particularly pronounced in the 1980s which was indicated by several authors (Milin et al. 1988; Karadžić & Milijašević, 2005). Although based on only two plots, our data also indicate that the problem of oaks drying is still present today, which indicates the need to investigate the causes of this phenomenon and develop measures to improve the current situation. It is not possible to develop appropriate measures needed to achieve sustainable forest management without analyzing the causes of poor forest condition and monitoring forest condition (Fabiánek et al., 2012). Continuous monitoring is a prerequisite for understanding the state and changes that occur in forest ecosystems as a consequence of the action of various harmful factors and is the basis for the development and implementation of protection measures. The number of plots with intensive monitoring should be increased in order to obtain even more reliable data and a better picture of the state of forests and the changes that occur in them as a result of changed environmental conditions around us.

Conclusions

Significant defoliation of tree crowns were found in sessile oak and pedunculate oak trees on the observed plots. The results indicated a pronounced influence of insect attacks on tree crowns defoliation and its variation. In both species, drying of part of the evaluated trees was recorded for ten years, in sessile oak 7.5% and in pedunculate oak 12.5% of evaluated trees dried up. The problem of oaks drying is continuously present, which indicates the need for detailed research of the causes of this phenomenon and finding measures to stop it and improve the current situation. It is necessary to establish additional plots with intensive monitoring in order to more reliably determine and quantify the causal relationships between harmful factors and the resulting damage of forest ecosystems.

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ESTIMATING INDICES OF SPATIAL FOREST STRUCTURE ON DIFFERENT TYPES OF SAMPLES ON SAMPLE PLOTS IN SERBIA

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Abstract

As a consequence of different influences, all changes in the forest are reflected through spatial structure. Indices of spatial forest structure quantify the condition, spatial relations, and changes in stand. The aim of this study was to examine the conditions of the application of spatial indices. In this study data from four equal sample plots in Serbia were used. Each sample plots was 0,20 acres in size. Spatial patterns of tree position in stands of Serbian spruce, Macedonian pine, Beech and Hungarian oak were investigated. Indices based on structural groups of reference trees and the nearest neighbors (*4*) were used for the analysis (*CE, Wi, Mi, Td, Ud*). The samples were divided into two groups. The first, control group, consisted of samples with the inclusion of the nearest neighbors located close to the plot boundaries, *plus sample*. The second group consisted of samples without additional recording, *minus sample*. Differences were tested at a significance level of 5% by the appropriate test (Paired T-Test). The analysis results show a statistically significant difference of the values of the spatial arrangement individual (*CE, Wi*) based on different samples. Other spatial structure indices (*Td, Ud, Mi*) does not shows a statistically significant difference. Indices of the spatial pattern of trees without additional recording (*CE', Wi'*) show higher values than indices from control group on all sample plots. Other index groups do not show a clear direction in relation to the type of samples. When determining the spatial structure index (*CE, Wi*) it is necessary to make edge-corrections with some of the existing methods. Therefore, for these indices, it is necessary to perform additional recording of trees outside the boundaries of the sample plots.

Key words: *indices of spatial forest structure, edge-corection, forest structure, Serbia.*

Introduction

As a consequence of different influences, all changes in the forest are reflected through changes in different structural relations in the horizontal and vertical sense. One of the most reliable parameters for monitoring changes are spatial structure indices. They quantify the condition, spatial relationships, and changes in the stand condition. Their advantage is that they quantify different structural elements simply and clearly. Diversity parameters that include the spatial distribution of individuals are the most reliable parameters for monitoring changes in forest ecosystems. These include indices of the diversity of tree positions (spatial pattern), tree species ratios, and dimensional diversity (Albert, Gadow 1999).

As indices are most often obtained from a sample, due to the impossibility of inventorying complete sets, it is necessary to establish clear criteria for their establishment. Spatial structure indices are used to monitor the spatial distribution of trees most often on experimental areas of limited size (Pommerening, Stoyan, 2006). Spatial structure indices, in contrast to the simple structure index, are burdened with an error that is the result of the assessment of the nearest neighbors by trees located on the peripheral parts of the sample plots (Matovic, 2012). It should be noted that the most precise solution was obtained with the *plus sample* (Stoyan, Stoyan, 1994). The *plus sample* implies periphery located trees on the experimental areas with determined their real neighbors, including those located outside of the

sample plots. The *plus sample* has no error resulting from the edge effect but requires additional measurements.

There are several methods of edge-correction, but NN_1 and NN_2 methods are the most often used indices developed on the concept of closest neighbors. The effects of edge-correction methods are closely related to the algorithmic structure of the indices used and the character of the spatial pattern of the trees. It should be noted that some edge-correction methods lead to a larger error than in the case of complete ignoring of the edge effect (Pommerening, Stoyan, 2006).

In practice, the most commonly used spatial structure indices are the parameters of individual trees based on the relationships of neighbor trees to describe the structure of small areas (Pommerening, 2002). A structural group with a center on a particular tree usually consists of four (Pommerening, Stoyan, 2006, Matovic, 2012) or three (Pastorella, Paletto, 2013) nearest neighbors.

To improve the use of structural indices and draw clear conclusions in the analysis of structural relations, is necessary to establish the conditions for their application.

Material and method

Data from four equal sample plots in Serbia were used in this study. The experimental areas are located in different localities (Figure 1) in different stand. On the mountain Tara in the stand of Serbian spruce (*Picea omorika*), on the slopes of Prokletije (Draga mountain) in the stand of Macedonian pine (*Pinus peuce*), on the mountain Goc in the stand of Beech (*Fagus moesiaca*) and on the mountain Cer in the stand of Hungarian oak (*Quercus frainetto*) (Table 1).

Table 1. Basic data of researched sites in Serbia

Sample plots	Locality	P (ha)	Elevation (m)	Slope (°)	Aspect	N	E
Serbian spruce	Tara	0,2	1.080	45	NW	43°55'35,19"	19°17'05,25"
Macedonian pine	Draga mt.	0,2	1.930	32	N	42°50'54,74"	20°21'06,52"
Beech	Goc	0,2	780	17	NE	43°33'48,06"	20°54'02,59"
Hungarian oak	Cer	0,2	170	15	W	44°39'21,53"	19°25'15,91"

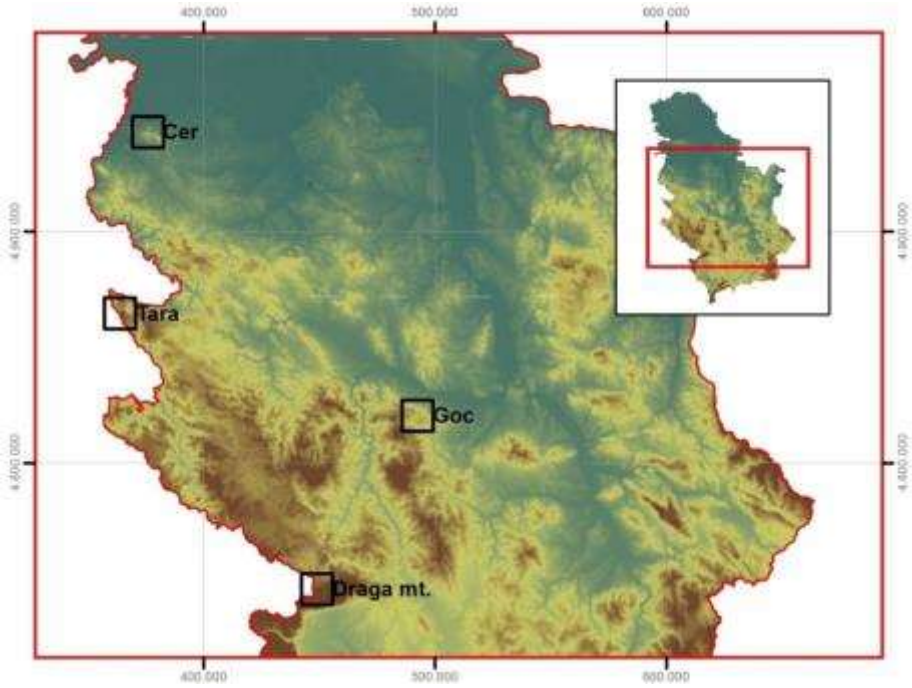


Figure 1. Schedule of sample plots in Serbia

Each sample plots was 0,20 acres in size, and square in shape. In each plots, the values of diameter at breast height ($d_{1,3}$), tree species, and position of trees were determined. The position of the trees was determined based on distance measurements (laser rangefinder *SNWAY 100*) and angles from the corners of the sample plots (*TO5 Wild* theodolite).

To examine the structural indices, the values obtained based on two samples were tested. The first, control group, consisted of samples with the inclusion of the nearest neighbors located close to the plot boundaries. *Plus sample* with additional recording. The second group consisted of samples without edge-correction, based on a sample of trees within the boundaries of the sample plots. *Minus sample* without additional recording (Figure 2). Indices based on structural groups of reference trees and the nearest neighbors (the four nearest trees) were used for the analysis. The structural indices of the spatial pattern of trees, the *CE* index of aggregation (Clark, Evans, 1954), the angular measure *Wi* (Gadow et al., 1998), the spatial relationship index between different species, the `species mingling` index *Mi* (Fueldner, 1995) and the *Td* differentiation (Fueldner, 1995) and the *Ud* dominance of diameter (Hui, et al., 1998) indices were analyzed.

The CRANCOD (Pommerening, 2012) software was used for data processing and calculation of the spatial structure index of trees.

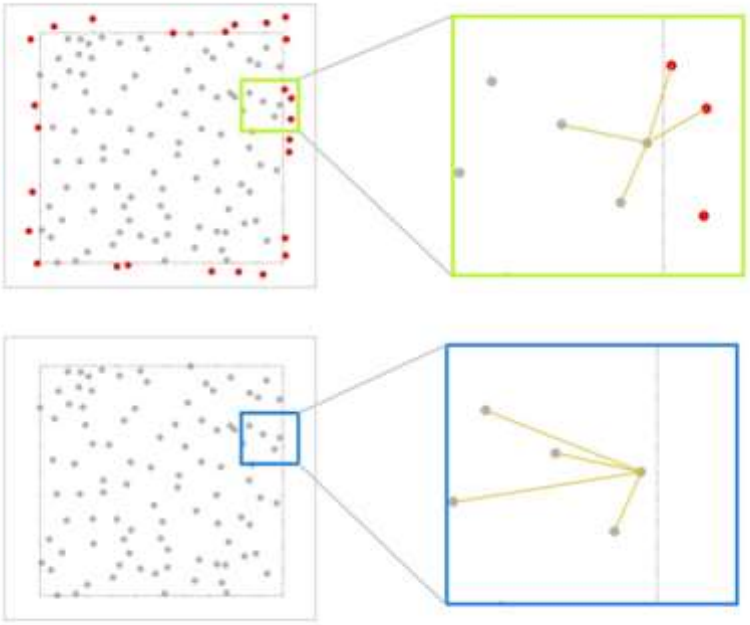


Figure 2. Analyzed sample types, *plus sample* with additional recording (upper) and *minus sample* without additional recording (below)

Differences between index values obtained from different samples were tested at a significance level of 5% by the appropriate test (Paired T-Test) in JASP statistical program. Testing the normality of data distribution was a condition for using the appropriate test.

Results and discusion

Testing of the analyzed indices based on different samples was performed on sample plots of different characteristics. The basic taxation values, diversity and spatial structure indices based on *plus samples* are as follows (Table 2).

The values of the number of trees per hectare established on the sample plots range from 325 (Macedonian pine) to 1.280 (Serbian spruce). The mean value of the diameter at breast height ($d_{1.3}$) is from 22.6 cm (Hungarian oak) to 39.7 cm (Macedonian pine). The Gini coefficient ($d_{1,3}$) shows the highest values in the sample plot of Macedonian pine (50.6%) and the least in the sample plot of Beech (28.6%). The sample plots have a different effective number of species, from 1.0 in the pure stand of Beech to 2.3 in the mixed Serbian spruce community.

Table 2. The basic taxation values, diversity and spatial structure indices

Sample plots	N·ha^{-1}	$d_{1,3}$ (cm)	Gini (%)	ENS*	CE	Wi	Mi	Td	Ud
Serbian spruce	1.280	23.6	37.8	2.3	1.07	0.57	0.36	0.31	0.49
Macedonian pine	325	39.7	50.6	2.1	0.91	0.60	0.42	0.34	0.47
Beech	480	23.8	28.6	1.0	1.32	0.55	0.00	0.24	0.53
Hungarian oak	525	22.6	42.2	1.7	1.19	0.56	0.23	0.34	0.50

*Effective Number of Species

According to the values of the *CE* index of aggregation, Macedonian pine (0.91) show a group arrangement of trees, while Beech trees (1.32) show the regular arrangement of trees in space. The values of the *CE* index of aggregation around 1.00 (Serbian spruce, Hungarian oak) indicate a random arrangement of trees in space. The determined values of the angular measure (*Wi*) confirm the previously stated claims that the tendency towards grouping is

shown by Macedonian pine (0.6). The sample plot of Beech show regular spatial pattern of tree positions (0.5). Values from 0.5 to 0.6 at the used standard angle of α_o=90° (Albert, 1999) confirm the random arrangement of trees (Serbian spruce, Hungarian oak).
Species mingling index (*Mi*) ranges from 0.00 for a pure Beech stand, to 0.42 for a mixed Macedonian pine stand.
Differentiation of trees by diameter (*Td*) is weak on the sample plot of Beech (0.24), while on the other three sample plots it shows medium values (0.31-0.34). The values of the diameter dominance index (*Ud*) are concentrated around the mean value (0.50) without significant deviations.
The results of the conducted testing of the researched indices *(CE, Wi, Mi, Td, Ud)* are presented below.
The CE index of aggregation describes the spatial structure. This index represents the relationship between the average measured distance of a tree and its nearest neighbor and the average distance when the trees are randomly distributed on the test surface (Clark, Evans, 1954). The tested values of the *CE* index of aggregation obtained based on two different samples show the following results (Table 3).

Table 3. Results of testing difference the *CE* index of aggregation

Sample plots	T	df	*p*	Mean Difference	SE Difference
Serbian spruce	-1.749	255	0.081	-0.023	0.013
Macedonian pine	-2.087	64	0.041	-0.074	0.035
Beech	-0.372	95	0.710	-0.051	0.137
Hungarian oak	-2.309	104	0.023	-0.118	0.051

On the sample plots of Serbian spruce and Beech are obtained values of the index which does not have a statistically significant difference. The value ***p*** is greater than the limit value (0.05) of statistical significance. On the sample plots of Macedonian pine and Hungarian oak are obtained values indicating statistically significant differences between the indices. The obtained values of the mean difference between the indices are of one-way character (negative values). This fact indicates that the values obtained based on the control *plus sample* are always less than the values obtained based on the *minus sample*.
The angular measure (Winkelmass) *Wi* describes the degree of regularity of the spatial distribution of the nearest trees in relation to the reference tree. The angular measure is calculated from the ratio of the actual values of the angles between adjacent trees and a predefined standard angle (Gadow et al., 1998). On all sample plots, the obtained values of the angular measure from different samples show a statistically significant difference (Table 4). This is shown by the value of ***p*** coefficients that are less than the limit value (0.05) of the analyzed statistical significance.

Table 4. Results of testing difference the angular measure *Wi*

Sample plots	T	df	*p*	Mean Difference	SE Difference
Serbian spruce	-2.641	255	0.009	-0.015	0.006
Macedonian pine	-2.009	64	0.049	-0.035	0.017
Beech	-2.574	95	0.012	-0.034	0.013
Hungarian oak	-3.445	104	< .001	-0.050	0.015

Also, as with the previous *CE* index of aggregation, the obtained values of the mean difference between the indices are of a one-way character (negative values). The values of the index obtained based on *plus sample* are less concerning the values of *minus sample*.

Species mingling Mi is a measure of spatial segregation, mixing of tree species. The species mingling is defined as the share of other species of the nearest neighbors concerning the reference tree (Fueldner, 1995). The results of the conducted testing (Table 5) in all sample plots do not show a statistically significant difference in the values of the index.

Table 5. Results of testing difference the species mingling *Mi*

Sample plots	T	df	*p*	Mean Difference	SE Difference
Serbian spruce	-0.277	255	0.782	-0.001	0.004
Macedonian pine	-1.298	64	0.199	-0.019	0.015
***Beech**	/	/	/	/	/
Hungarian oak	-1.000	104	0.320	-0.005	0.005

*pure stand

The values of the mean difference between the analyzed parameters are one - way. The values of the *plus sample* are smaller concerning the obtained values of the *minus sample*. The sample plot of the Beech stand was excluded from the analysis of the species mingling, it is a pure stand.

The differentiation index T describes the spatial distribution of tree dimensions. This most often refers to the spatial distribution of the values of diameter at breast height (Fueldner, 1995) as is the case in this analysis. The results do not show a statistically significant difference in any of the analyzed sample plots (Table 6).

Table 6. Results of testing difference the diameter differentiation index Td

Sample plots	T	df	*p*	Mean Difference	SE Difference
Serbian spruce	1.175	255	0.241	0.001	0.001
Macedonian pine	-1.137	64	0.260	-0.009	0.008
Beech	-1.814	95	0.073	-0.014	0.007
Hungarian oak	0.776	104	0.439	0.003	0.004

The values of the mean differences between the diameter differentiation indices are in different directions. The values of positive differences were obtained in the sample plots of Serbian spruce and Hungarian oak, while negative values were determined in the sample plots of Macedonian and Beech.

The dominance index U quantifies the share of neighboring trees that have larger dimensions (breast diameter, height, etc.) concerning the reference tree (Hui, et al., 1998). The index of diameter at breast height dominance (*Ud*) was used in this analysis. The results show (Table 7) that the values based on the conducted testing do not show statistically significant difference concerning the obtained values of the coefficient ***p*** (greater than 0.05).

Table 7. Results of testing difference the diameter dominance index *Ud*

Sample plots	T	df	*p*	Mean Difference	SE Difference
Serbian spruce	-0.577	255	0.565	-0.002	0.003
Macedonian pine	-1.150	64	0.254	-0.019	0.017
Beech	0.516	95	0.607	0.013	0.025
Hungarian oak	-0.631	104	0.530	-0.005	0.008

In the sample plots of Serbian spruce, Macedonian pine and Hungarian oak, negative mean values of the differences between the indices were obtained, while in the sample plot of Beech, a positive value was obtained. As the values of different types of samples are of different orientation, we can attribute them to a random distribution of diameter dominance.

This refers to trees that enter the index structure in the *plus sample* and are omitted in the *minus sample*.
In general, the results show that different values obtained on the basis of different types of samples are a consequence of the algorithmic structure of the analyzed index and the random distribution of diameter values in space (*Ud, Td, Mi*). While for certain indices (*Wi, CE*) it is a consequence of different input data, the differences of which are one-way.

Conclusion

According to the values of the *CE index of aggregation* and *Wi angular measure* Macedonian pine show a group spatial pattern of tree positions, while Beech trees show the regular arrangement of trees in space. Serbian spruce and Hungarian oak sample plots show a random arrangement of trees in space.
Based on the presented results, we can conclude that the values of the spatial distribution index of individuals (*Wi, CE*) determined based on different samples are statistically significantly different. This statement especially refers to the *angular measure Wi* where the differences were found in all sample plots. Other groups of structural indices that include spatial distribution (*Td, Ud, Mi*) do not show statistically significant differences in any of the studied situations.
It was found that the spatial distribution indices of individuals (*CE, Wi*) established based on *plus sample* show lower values than the index *minus sample* on all sample surfaces. Other groups of structural indices do not show a clear direction concerning the type of samples.
The differences in the values of the indices were due to the algorithmic structure of the index and the random arrangement of edge trees on the sample plots (*Ud, Td, Mi*), and different input data of one-way character (*Wi, CE*).
The occurrence of errors in establishing the index can be corrected by introducing some of the edge-correction methods. The importance of obtaining accurate values of the spatial structure index is exceptional. Spatial structure indices are of great importance in the long-term observation of changes (monitoring trends) in the structure of the spatial distribution of trees. Empirical amounts of errors lead to misinterpretation of trends and drawing bad conclusions about the change in the situation.
When determining the spatial structure index (*CE, Wi*) it is necessary to make edge-corrections with some of the existing methods. Therefore, for these indices, it is necessary to perform an additional recording of trees outside the boundaries of the sample plots in order to obtain accurate data. However, a large number of indices are most often used in spatial structure analyzes. Accordingly, it is necessary to establish their values on a single sample. As statistically significant one-way errors occur in some indices (*CE, Wi*), it is necessary to collect data on the basis of a *plus sample* or by using some of the edge-correction methods.

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ANALYSIS OF ECOLOGICAL-COENOLOGICAL VITALITY OF BLACK PINE AND RED OAK ON HABITAT OF FORESTS TYPE OF TURKEY AND VIRGILIANA OAK IN THE PARK-FOREST IN BELGRADE (SERBIA)

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Abstract

Considering the specific purpose of park-forest "Košutnjak" in Belgrade (Serbia), originating from spatial-orographic location and prioritized direct needs of citizens of Belgrade for multifunctional content, related to various ecological, social and wellness functions, as well as its subsequent functional planning projection and its legal regulations, park-forest "Košutnjak" has been organized according to plan into three prioritized functional units that have been established based on evaluation and estimate of valorization elements related to concrete purpose. In that regard, the largest surface area is projected to be related to the social function as the most prominent function, based on immediate needs of citizens of Belgrade, and especially based on valorization elements that are related to providing complete content. In accordance with its purpose, these functional units contain largest number of allochthonous and autochthonous tree species, related to primary typological habitats, as well as species belonging to secondary habitats. Considering all these, projection of this research was primarily aimed towards functional sustainability of concrete purpose and therefore, ecological-coenological vitality of black pine and red oak on habitat of primary forest type of Turkey and Virgiliana oak (*Orno-Quercetum cerridis-virgilianae*) on pararendzina was analyzed. Red oak (*Quercus rubra L., Q. borealis Michx f., Q. borealis* (*Marsh.*)) on this habitat of primary forest type exhibits significant ecological-coenological dominance, compared to black pine. At the same age, red oak achieves significantly larger production effects and is more stable in ecological-coenological sense. Dynamism of succession differs, that is, black pine is being suppressed by primary types of species (edificators). This is an important insight for projection of following functionally sustainable management plans. That mostly pertains to validity of future planned actions in regards to black pine on this type of forest habitat.

Key words: *Red oak, black pine, ecological vitality, coenological vitality, functional sustainability.*

Introduction

The existence of a forest does not in itself provide functionality in value structure and functional multipurpose sustainability. That is, the value effects of the forest observed from the point of view of public utility functions, can be achieved in the first phase through methodical zoning (determining the basic purpose of the forest), and later the development of planning modalities (ecological-functional planning) within various forest complexes and specifically parts of the forest complex through emphasizing the planned purpose and functional sustainability.

The forest, as the most perfect form of nature's and historic activity, represents the most important natural basis of life. The value of forest ecosystems nowadays is not related to the quantity and quality of wood volume, but the value of the forest as the most important object

for oxygen production, protection from the soil erosion, existence of water sources, microclimatic effects, health effects, etc. (Milošević, 2012).
In order to fully create the planning basis and achieve the full multi-purpose-functional content of forests, it is necessary to create realistic assumptions of different character, depending on the specific function.
Therefore, each dedicated unit is characterised by planning requirements in terms of sustainability of functional durability.
In specific research, the recreational function of forests in the forest park "Košutnjak" is emphasized as the most spatially dominant and most pronounced in terms of the needs of the city of Belgrade. Accordingly, this dedicated unit is also the subject of *multidisciplinary research* and approach in evaluation and planning settings, with the aim of the most optimal provision of the needs of the inhabitants of Belgrade, given the spatial and orographic position of this forest park.
This determined the aspect of this research, having in mind the value elements and their assessment in terms of evaluating the recreational function, as well as creating a starting point for their realization.
One of the essential aspects is the ambient structure expressed through the representation of species and their structure. Starting primarily from that, planning concepts are related to the representation of a larger number of species, both autochthonous and allochthonous. This required the introduction of a large number of species into the habitats of different types of forests. The primary typological structure in the "Košutnjak" forest park is very pronounced.
The entire complex-park of forests "Košutnjak" is expressed through the definition of eight types of forests of primary ecological-vegetation structure (Jović et al. 1994). Different species show different ecological-coenological vitality in the habitats of different forest types. The primary function of the forest park "Košutnjak" and the functional ecological-coenological and functional optimization of the recreational function of this part of the forest complex was decisive in the setting and goal of this research.
Thus, the aim of this research is to investigate the ecological-coenological vitality of red oak and black pine on the same type of forest, that is, forest type of Turkey and Virgiliana oak (*Orno-Quercetum cerridis-virgilianae*) on pararendzina soil and their further planning orientation, all with the aim of sustainability and improving the optimization of the recreational function.

Materials and metods

The research covers the forest park "Košutnjak", in capital of Serbia - Belgrade, which is a complex of a specific character arising from the spatial and relief-orographic position and overall potentials, in terms of multifunctional provision of the needs of Belgrade residents for different facilities and environmental quality.
According to its geographical position, managing unit "Košutnjak" is located between 44°46'52"-44°44'40" north latitude and 20°25'40"-20°28'05" east longitude (east of Greenwich).
The altitude range is from 75 m to 217 m above sea level, with a very pronounced orography of the terrain (Figure 1.).
Forest park "Košutnjak" is in the most immediate, urban part of the city of Belgrade, in spatial terms. The relief-orographic position is the result of refraction and influence of two relief regions, which directly produced and reflected on the specific and primary typological composition of this forest, which is expressed through a very pronounced and heterogeneous typological and primary structure of forest types and their characteristics.

In the forest park "Košutnjak", eight types of forests were investigated and defined and they are very different in regards to the first degree of typological classification of forests or the complexes of forest types, with a dominant representation of forest types of xero-mesophilic and xero-thermophilic character (Jović et al. 1994). Forest type of Turkey and Virgiliana oak (*Orno-Quercetum cerridis-virgilianae*) on pararendzina soil (Jović et al. 1994) on which many different types of allochthonous and autochthonous species can be found, is substantially represented in the forest park „Kosutnjak", especially in the part of area with a priority recreational purpose.

Due to its functional priority and multifunctional use and purposeful zoning, a large number of allochthonous tree species are represented in the "Košutnjak" forest park.

For the analysis of the mutual ecological-coenological relationship, artificially raised stands of red oak and black pine in the habitat of the primary type of Turkey and Virgiliana oak (*Orno-Quercetum cerridis-virgilianae*) forest on pararendzina soil, were included. The basic determination for this analysis is the presence of black pine in the total area of the forest park "Košutnjak" as well as in this purposeful unit, on the one hand, and the specific bio-ecology of these species, especially red oak in terms of adaptability to habitat characteristics of this forest type, on the other hand, and functional vitality, which allows a realistic view and the initial basis in the planning bases and priorities of functional planning in relation to other species in this type of forest and the species that make up the structure of the primary forest type. Artificially raised stands of red oak and black pine are 50 years old.

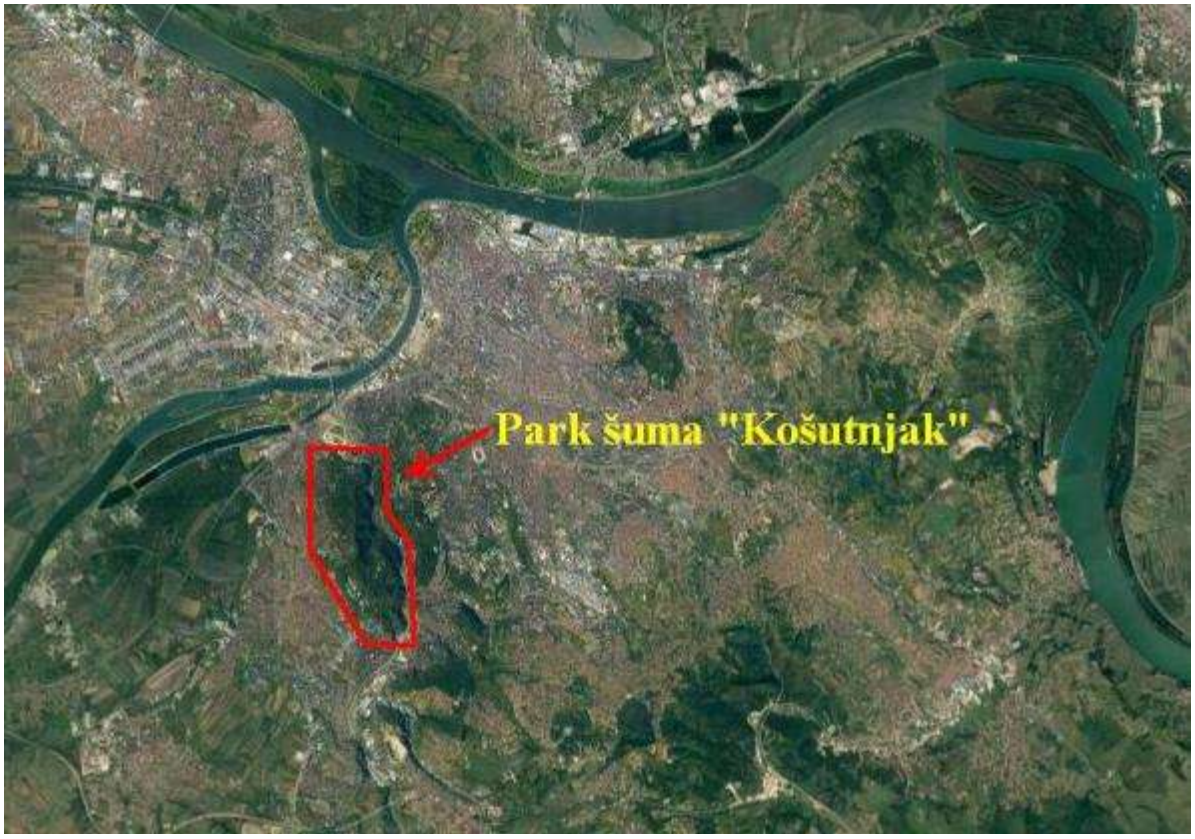


Figure 1. Geographic position of Košutnjak (Bićanin, 2020.)

The research is in adjustment to the methodological procedure suitable for this type of research (Milošević, 2012). The basic starting point was phytocenological research.

To characterise the cenological relationship of species in artificially raised stands on the forest type of Turkey and Virgiliana oak (*Orno-Quercetum cerridis-virgilianae*) on pararendzina soil, floristic composition analysis was used.

Phytocenological images made by the standard Braun-Blanquet method were used for the analysis of the floristic composition of the investigated areas. (Braun-Blanquet, 1964). Syntaxon names are given according to Tomić, Rakonjac, (2013).

Results and Discussion

Black pine and red oak in artificially raised stands on forest type of Turkey and Virgiliana oak (*Orno-Quercetum cerridis-virgilianae*) on eutric brown soil on lessive in Košutnjak, show significant disparity in the course and dynamism of ecological-coenological vitality, that is, ecological-coenological flow and development towards progressive succession.

Namely, in the case of an artificially raised black pine stand at the age of 50 years (Figure 2), a very pronounced course of progressive succession is evident, that is, the establishment of the structural development of the primary type of forest. In the first floor, an identical presence is evident in terms of the number and stand crown cover of both black pine and Virginian oak, edificator of the primary forest type (*Quercus virgiliana*) (Table 1, phyto-cenological image 1/19).

Also, the presence in the first floor of the characteristic second edificator of the primary type of forest, Turkey oak (*Quercus cerris*), is evident.

In the second and third floors, the primary identification structure of the representation of characteristic and differential species of the primary forest type was ascertained - forest type of Turkey and Virgiliana oak (*Orno-Quercetum cerridis-virgilianae*) on pararendzina soil, particularly pronounced differential species characteristic of the primary forest type that dominate in number and stand crown cover in the primary forest type, that is, forest type of Turkey and Virgiliana oak (*Orno-Quercetum cerridis-virgilianae*) on pararendzina soil (*Glechoma hirsuta, Geum urbanum*) (Tomić et al. 1994).



Figure 2. Artificially raised stand of black pine aged 50 years in the habitat of the forest type of Turkey and Virgiliana oak (*Orno-Quercetum cerridis-virgilianae*) on pararendzina soil-forest park "Košutnjak" (Bićanin, 2020.)

Red oak in the habitat of this type of forest has significant ecological and coenological differentials in relation to black pine.
At the identical age (50 years), red oak in an artificially raised stand in the habitat of this type of forest shows ecological and coenological stability and complete dominance, that is, at the stated age there is complete absence of edificators of the primary forest type (Figure 3). In the first floor, the absolute dominance of this species is evident, with a complete stand crown cover and imposing dimensions of trees and overall high production effects.



Figure 3. Artificially raised red oak stand aged 50 years on the habitat of the forest type of Turkey and Virgiliana oak (*Orno-Quercetum cerridis-virgilianae*) on pararendzina - forest park "Košutnjak" (Milošević, 2020.)

Cenological stability of red oak in the habitat of this type of forest is especially pronounced in the third floor (Table 1). Namely, in this third floor, which serves as identification floor especially at more pronounced stand ages and different bioecology of a specific species in habitats of different forest types, the number and stand crown cover of differential species of primary forest type is less pronounced, which is characteristic of black pine in this forest type. What especially differentiates the ecological-coenological stability of red oak on this type of forest in relation to black pine, is the dominance of this species in the third floor, which in relation to other species (that are characteristic to primary composition) is more pronounced in number and stand crown cover.
Red oak in the habitat of this type of forest, namely, on forest type of Turkey and Virgiliana oak (*Orno-Quercetum cerridis-virgilianae*) on pararendzina soil and in relation to other species in artificially raised stands on the type of forest of identical coenoecological affiliation - complex of xero-thermophilous Hungarian and Turkey oak forests types (Jović, et al. 2009)

- forest type of the Hungarian and Turkey oak (*Quercetum frainetto-cerridis typicum*) on brown lessive soil, shows identical cenological-ecological vitality and stability in question and other species of deciduous and coniferous trees (Bićanin, 2015a, 2015b; Milošević 2012, 2016, 2017).

Table 1. Phytocoenological table of studied stands

Association	***Orno-Quercetum cerris-virgilianae* B. Jovanović & Vukićević 1977.**		
Locality	Košutnjak		
Relevé number	1/19	2/19	
Section	3a	4a	
Relevé area (m²)	400	400	
Altitude (m)	115	130	
Aspect	NE	NE	
Slope(◦)	5	5	Presence constancy
Parent material	lessive	lessive	
Soil	eutric brown (haplic cambisol)	eutric brown (haplic cambisol)	
LAYER I			
Canopy	0.8	0.9	
Middle height (m)	20	22	
Middle distance (m)	4	5	
Quercus virgiliana	2.2		
Pinus nigra	2.2		
Tilia tomentosa	1.1		
Prunus avium	+	1.1	
Quercus cerris	+		
Quercus rubra		4.4	
Prunus serotina		+	
LAYER II			
Canopy	0.7	0.3	
Middle height (m)	1	4	
Middle distance (m)	1	2	
Cornus sanguinea	+	+.2	
Acer campestre	+.2	+	
Prunus avium	+	1.1	
Tilia tomentosa	1.2		
Crataegus monogyna	+.2		
Ulmus minor	+.2		
Fraxinus ornus	1.1	+.2	
Sambucus nigra	1.1		
Prunus avium	+		
Prunus cerasifera	+	+	
Ulmus campestris		+	
Cornus mas		+.2	
LAYER III			
Cover	0.9	0.8	
Hedera helix	2.2		

Fraxinus ornus	+.2	2.2	
Urtica dioica	2.2		
Geranium robertianum	2.3	1.2	

Continuation of the table 1.

Glechoma hirsuta	2.2	1.2	
Mycelis muralis	+		
Juglans regia	+	+	
Galium aparine	1.2	1.2	
Lonicera caprifolium	1.1	+.2	
Acer pseudoplatanus	+		
Geum urbanum	1.1	1.2	
Alliaria officinalis	+.2	+.2	
Tilia tomentosa	+.2	+	
Clematis vitalba	+.2	+.2	
Viola sylvestris	+.2		
Mahonia aquifolium	+.2		
Dactylis glomerata	+.2	1.2	
Quercus rubra		2.2	
Hypericum perforatum		+.2	
Prunella vulgaris		1.1	
Taraxacum officinale		+	
Brachypodium sylvaticum		+.2	
Quercus cerris		+.2	
Acer campestre		+.2	
Prunus avium		+	
Cardamine bulbifera		+.2	
Acer tataricum		+	
Polygonatum odoratum		+.2	
Aristolochia clematitis		+.2	
Carex divulsa		+.2	
Lapsana communis		2.2	
Viola alba		1.2	
Poa trivialis		1.2	
Rosa gallica		+.2	
Stellaria media		+.2	

Conclusion

Concrete research has created realistic assumptions in further planning and functional sustainability when it comes to these two species, i.e. red oak (*Quercus rubra L, Q. borealis Michx f., Q. borealis* (*Marsh.*)) and black pine (*Pinus nigra*) on the habitat of forest type of Turkey and Virgiliana oak (*Orno-Quercetum cerridis-virgilianae*) on pararendzina. This especially refers to the planning guidelines which are exclusively related to the functional sustainability and protection of various structural contents and overall environmental protection. This implies a different planning approach in the next ten years of planning when it comes to these two types.

Red oak is significantly ecologically and coenologically more vital in relation to black pine in the habitat of this type of forest, which directly reflects on the further planning approach and immediacy of planning procedures in the next planning procedure - the planning period.
In the next planning half-period - planning period, and guided primarily by the functional ecological and coenological vitality and sustainability of this dedicated unit, these two species on the basis of these studies require a different planning approach. With red oak, a regular planning procedure and extension of the functional felling cycle (infrastructural ecologically specific effects of the forest on a specific type of forest) should be carried out.
When it comes to black pine, it is necessary to revise the planning procedure in the following functional plans, that is, to start the process of restitution and establishing the structure of the primary type of forest.

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MORPHOLOGICAL CHARACTERISTICS AND CONTENT OF HEAVY METALS IN FRUITS OF DIFFERENT BLUEBERRY VARIETIES

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Abstract

The research was conducted in a blueberry plantation in the village Božurnja situated in the western part of Serbia. The paper deals with morphological parameters of the blueberry fruits such as: medium fruit diameter (mm), fruit mass in fresh and dry condition (g), respectively, and water content in the fruit (%), as well. Content of some heavy metals in the fruits such as Zn, Cu, Pb, Cd, Fe, Mn has been also examined. Statistical processing of collected data was performed according to descriptive statistics, one factor analysis of variance and LSD-test. The aim was to determine fruit quality of two blueberry varieties (*Duke* and *Huron*) based on above mentioned elements. As for recorded heavy metals, their content was very low and therefore with no significant effect on the quality of fruit. Among all researched heavy metals, the greatest concentration was recorded for Fe, while the least was recorded for Cd. Low concentrations of Zn and Mn, as the elements with a significant role in all physiological processes in the plants, could affect a bit yield of the fruits. Based on the obtained results, it can be deduced that *Duke* has larger fruit than *Huron* variety. All in all, it can be said that quality of the fruits of both analysed blueberry varieties is satisfactory. Having in mind that blueberry fruits have a lot of medical properties, it is necessary to establish more plantations with this useful species.

Keywords: *blueberry varieties, Božurnja, heavy metals, fruit characteristics*.

Introduction

The species of the *Vaccinium* genus include deciduous or evergreen shrubs which belong to the family *Ericaceae*. They are located in the mountanious regions of central Europe and in the boreal and sub-artic regions of northern Europe, particularly on the ground floor of coniferous forests. In the hilly and mountanious regions on the territory of Serbia, three species of this genus are described: *V. myrtillus*, *V. uliginosum* and *V. vitis-idaea* (Bjedov, 2012).

Blueberry has a lot of medical properties, especially its leaves and fruits (Sarić, 1989; Bown, 1995; Chevallier, 1996). Taxa from *Vaccinium* genus are considered as plants with a great contribution to aesthetic landscapes characteristics. Lowbush forms are used for forming of attractive ground covering (Kirkman and Ballington, 1985). Shrub forms of the genus are very attractive in the landscape, particularly with their spring flowers (Dirr, 1990).

On the territory of Serbia, growing needs for blueberry can not be satisfied just by collecting in the nature, because it endangers its survival. Therefore is necessary to establish bluebbery plantations as the only longterm and sustainable solution (Stepanović and Radanović, 2011). Blueberry planting and its planned breeding by hibridization started relatively late. Planting of highbush form of American blueberry in Serbia occured a few years ago (Mratinić, 2015).

Although there is no precise data about the areas with this culture in Serbia, in the last decade was established about 100 ha of new plantations of highbush form, mainly in western Serbia, where its production reaches about 100 t a year (Milošević *et al.*, 2015).

Duke variety was made in Maryland by crossing *Ivanhoe x Earlyblue* varieties. It blooms late, which is why it is recommended for areas where late frosts occur. It is a very fertile variety with an average yield per bush of 6 kg. The fruits can be preserved in a fresh condition for a long time and tolerate transport well. The variety is suitable for manual and mechanized harvesting.

Huron is a newer American, very productive variety. The shrub is lush and erect with numerous shoots of medium branching. In full maturity, its fruit has a superior and sweet taste. For that reason, it is recommended to avoid early harvesting as the fruits are very sour in that time.

Knowledge about the accumulation and toxic effect of heavy metals in plants is extremely important, because this is a possible way of their entering in the food chain (Kastori, 1997; Memon et al., 2001). The degree of heavy metals accumulation in plant tissue is determined by numerous biotic and abiotic factors, and among them, the genotype specificity is one of the most important (Pajević et al., 2008; Nikolić et al., 2008). High concentrations of heavy metals in plants result in numerous anatomical, morphological and physiological changes, while the accumulation of heavy metals in plant tissue indicate the important role of specific plants as bioindicators of environmental pollution (Stanković, 2008; Stanković et al., 2011). There are more than 400 plants known as hyperaccumulators of metals, which can accumulate high concentration of metals into their aboveground biomass. Based on Baker and Brooks (1989), hyperaccumulators are defined as plants that accumulate > 1.000mg/kg of Cu, Co, Cr, Ni or Pb, or > 10.000 mg/kg of Mn or Zn. It should be emphasised that damaging effects of heavy metals on an annual level exceed the overall damaging effects of radioactive and organic waste generated each year (Nriagu, 1979; Pacyna and Munch, 1989).

Plants develop different survival mechanisms in sites with high concentrations of heavy metals in the ground. Depending on their reaction to heavy metals presence, plants are divided into three groups: *excluders* - they avoid accumulation of heavy metals, *accumulators* - plants that accumulate a lot of heavy metals and *indicators*, whose concentration of heavy metals coincides with its environmental concentration (Bhargava et al., 2012). Plant metal uptake is influenced by soil factors including pH, organic matter, and cation exchange capacity as well as plant species, cultivars and age. The mobility and availabilty of heavy metals in the soil are generally low, especially when the soil is high in pH, clay and organic matter (Jung and Thornton, 1996; Rosselli et al., 2003).

The scope of the paper was to determine quality of fruits of two blueberry varieties based on their morphological features and heavy metals content, as well.

Material and methods

Study area

Collecting of examined material (fruits of both blueberry varieties) was performed during vegetation period in a blueberry plantation situated in Božurnja village, owned by the Maksimović farm. Božurnja is a village in the municipality of Topola, in the area of Šumadija, at the elevation of about 300 m.

Determining of fruit morphological characteristics

For both varieties (*Duke* and *Huron*) the sample, including 100 fruits, was collected during vegetation period. Performed morphological research on collected fruits included the analysis of the fruit diameter (mm), where two cross diameters D_1 and D_2 were measured by using

digital device to the nearest hundredth of a millimetar. During data processing, the average value of fruit diameter obtained from the ratio $(D_1+D_2)/2$ was taken.
Fruit mass (g) was measured for both varieties in fresh and dry condition. The mass was measured with an electric scale with an accuracy of one hundredth of a gram. Drying was performed in an oven for 48 hours at 68°C. Based on obtained mass (fresh and dry condition) was calculated water content in the fruit due to equtation: SV = (MS – MSU)*100/MS (Ivetić, 2013), SV – water content, MS – mass of fresh fruits, MSU – mass of dry fruits

Determining of heavy metals content in the fruits

Heavy metals content (Z_n, C_u, P_b, C_d, F_e, M_n) in the fruits of two blueberry varieties was measured by atomic absorption spectrophotometry. The analysis was performed with three replicates.

Statistical data processing

Statistical data processing was performed in programme package Statgraphics Centurion VI version. The paper presents the results of descriptive statistics: minimum and maximum value, mean value (x), standard deviation (SD), coefficient of variability (CV%), one-factor analysis of variance (ANOVA) and LSD test for morphological characteristics of fruits.

Results and discussion

A mean value of Duke variety fruit diameter was 13.20 mm, while Huron variety had a little bit lower value – 12.65 mm. Range of variation for this fruit characteristic was wider by Duke than by Huron (Table 1). P-value for this characteristic is less than 0.05, which shows there is a statistically significant difference between mean values (Table 1). Based on obtained results of LSD test, it can be deduced that two homogeneous groups were formed which shows statistically significant difference between calculated mean values (Table 1).

Table 1. Descriptive statistics, analysis of variance and LSD test for an average fruit diameter

	Medium fruit diameter (mm)			
Variety	**min-max**	**x**	**SD**	**CV**
Duke	8,6-16,89	13,1951	1,74509	13,2253%
Huron	9,12-15,87	12,6549	1,266	10,004%
Analysis of variance				
Between varieties		**Mean Sqare**	**F-ratio**	**P-Value**
		14,5908	6,28	0,0130
LSD тест				
Variety	**Mean value**		**Homogeneous group**	
Duke	13,1951		X	
Huron	12,6549		X	

Fruit mass in fresh and dry condition for both varieties, and water content in the fruit, as well, were measured two times (Table 2). A bit higher water content was recorded by Huron (87 %) than by Duke (85 %) variety (Table 2).
There were analyzed following heavy metals: Z_n, C_u, P_b, C_d, F_e and M_n. Based on the obtained results, the contents of the determined heavy metals can be arranged in the following descending order: $F_e>M_n>Z_n>C_u>P_b>C_d$. F_e was recorded with the highest concentration which varies between 20,82 and 25,36 mg/kg by *Huron*, and between 25,75 and 31,16 mg/kg by *Duke*, respectively. As for C_d, the most dangerous and toxic heavy metal, it almost was not recorded by *Duke*, while by *Huron* its content was very low (Table 3). If we compare mean values of detected heavy metals by both varieties, F_e and C_u are higher by *Duke*, unlike M_n, C_d and P_b, whose concentrations are higher by *Huron*. As for Z_n content, it is almost the same (Table 3). It should be emphasised that concentrations of all detected heavy metals are much less than their maximal allowed concentrations. Compared to heavy metals content by some medical herbs (Ranđelović, 2015), values obtained in this paper are about five time less.

Table 2. Fruit mass in fresh and dry condition (g) and water content in the fruit (%)

Fruit mass	Variety			
	Duke		Huron	
	I	II	I	II
Fruit mass (50 individuals) in fresh condition (g)	117	121	142	114
Fruit mass (50 individuals) in dry condition (g)	17,15	17,7	17,51	15,79
Water content in the fruit (%)	85,342	85,372	87,669	86,149

Table 3. Heavy metals content in the fruits of two blueberry varieties

Number of the sample	Variety	Heavy metals content (mg/kg)					
		Zn	**Cu**	**Pb**	**Cd**	**Fe**	**Mn**
1 I	**Huron**	5,6481	1,6285	0,5795	0,0266	25,3597	6,9802
1 II		5,3170	1,4534	0,6767	< nd	22,1948	6,7371
1 III		5,4111	1,9979	0,0033	0,0266	20,8185	6,5965
X		**5,5487**	**1,6933**	**0,4198**	**0,0177**	**22,7910**	**6,7713**
2 I	**Duke**	5,6608	2,8604	0,2797	< nd	31,1611	6,5199
2 II		5,4691	2,8044	0,2329	< nd	28,9155	6,4837
2 III		5,4437	2,6535	0,0667	0,0233	25,7517	6,4404
X		**5,5245**	**2,7728**	**0,1908**	**0,0078**	**28,6094**	**6,4813**

Stanković et al. (2015) researched Zn concentration in leaves of different plants in the mountain of Kosmaj (Serbia) and concluded that *Pinus nigra* and *Taraxacum officinale* are the greatest accumulators.

As for presence of Mn, Stanković et al. (2015) also investigated it on Kosmaj and found that its greatest concentration was in leaves by *Fagus sylvatica* and *Quercus petraea*.

Kabata-Pendias and Pendias (1992) established that metal concentrations in plants growing in uncontaminated soils were 0.3-18.8 and 1.1-33.1, for Pb and Cu, respectively, whereas the highest metal concentrations in plants growing in contaminated soils were 1506 and 1123 mg/kg for Pb and Cu, respectively.

Stojnić et al. (2019) investigated heavy metals content in foliar litter and branches of *Quercus petraea* and *Quercus robur* and concluded that concentrations of Mn and Fe were significantly higher in the foliage of both oak species, whereas slightly higher content of Zn and Cu was observed in the collected branch material.

Lacatusu R. and Lacatusu A-R. (2008) researched vegetable and fruits quality within heavy metals polluted areas in Romania and found that the medium values of total and mobile heavy metals content (Cd, Cu, Pb, Zn) in most samples exceed the maximum allowable limits. These outruns are up to 2.3 times (Cd), 1.7 times (Cu), 2.6 times (Pb) and 2.1 times (Zn) for the total content, 10.5 times (Cu) and 4,2 times (Cd), 10.5 times (Cu), 7.2 times (Pb) and 4.5 times (Zn) for the mobile forms content.

The most suitable soils for blueberry planting are acidic, carbonate-free and humus-rich soils. Research of fruits chemical composition of some highbush blueberry forms shows a big differences related to content of dry substances, sugar, total acids, total mineral substances, macro and micro elements, not only between different varieties, but also between researched years (Stanisavljević and Joković, 1987).

It can be said without exaggeration that the biological value of blueberries is much higher than any other food. This means that regular consumption of even small amounts of this fruit is quite a reliable remedy against the effects of many factors that endanger human health (Šoškić et al., 1997).

In edible plants, the limit given by the Word Health Organization for zinc is 27.4 ppm, iron 20 ppm, manganese 2 ppm, cadmium 0.21 ppm. According to Allaway, the copper content in

agricultural products should be between 4 and 15 ppm, while according to the limit of the World Health Organization it is 3 ppm and less (Ranđelović, 2015).

Conclusions

The paper deals with morphological features of the fruit of two blueberry varieties (Huron and Duke) such as: diameter, mass and water content. There was also recorded content of following heavy metals by both varieties: Zn, Cu, Pb, Cd, Fe, Mn. The scope of the paper was to determine quality of blueberry fruits based on above mentioned characteristics. Fruits are a bit larger by Duke, whereas a water content is a bit higher by Huron variety. Heavy metals content is much under maximal allowed concentrations for both varieties, which means there is no danger for human health. Based on the obtained results, we can deduce that Fe content was the highest, while Cd content was the lowest. Cadmium, as element with a very high toxicity and carcinogenicity, was almost not detected by both varieties. As for basic elements such as Zn and Mn, their low concentrations can affect a rate of growth and yield of both varieties, bearing on mind their essential importance for all physiological processes maintaining. Double concentration of Pb was recorded by Huron, unlike Fe, whose a bit more higher concentration was detected by Duke. As for the other heavy metals, there is almost no difference between researched varieties. Based on the obtained results related to the researched parameters of the fruit, as well as the fact that sale of blueberry from this area is constantly increasing, it can be stated that the quality of blueberries is more than satisfactory. In order to meet the growing needs for this medicinal and aromatic plant, it is necessary to establish new plantations that can enable mass production of this extremely useful plant.

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THE INFLUENCE OF THE GLOBAL CLIMATE CHANGE ON FORESTRY

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Abstract

Under the influence of energy exchange disturbances between space and the Earth, and the increase of greenhouse gases and thus accelerated global warming, climate changes out of its usual natural variability have been caused. The consequences of the speed of current climate change are the frequency and increase of extreme events (droughts, floods, fires, etc.). Mankind is fighting this problem by applying measures for ensuring sustainable development and preservation of the natural environment. Forest ecosystems are particularly sensitive to climate change due to their low speed of change and adaptation ("passive adaptation") to the increasingly pronounced xerothermization of the climate. At the same time, extremely hot and dry weather conditions are the cause of high sensitivity of vegetation: displacement of distribution zones of some species of plants and animals towards the north and higher altitudes, reduction of biodiversity, reduction of plant vitality, dieback and extinction of some species, etc. The risks arising from the negative effects of climate change are reflected in the financial losses due to the reduction of the quantity and quality of wood mass in forestry, as well as the degradation of the environment and normal functioning of the biosphere. Therefore, forestry, along with agriculture, water regime and the environment in general, is considered one of the key vulnerable sectors. Applying appropriate measures in the preservation and maintenance of forest ecosystems is necessary and urgent and requires long-term planning, based on a more reliable and precise definition of future climate change.

One of the key measures for adaptation, which requires long-term planning and is very sensitive to the reliability of vulnerability assessment in the conditions of changing climate is: selection of tree species and their varieties with greater tolerance to changed climate conditions, but also adaptability to optional climatic conditions and forest management approach with the aim of mitigating climate change ("active adaptation").

Keywords: *Climate change, Forestry, Vulnerability, Adaptation*

Introduction to the problem of climate change

Numerous researchers (Beniston *et al.*, 2007; Adams *et al.*, 2012; Smith *et al.*, 2011; Cavin *et al.*, 2013; IPCC, 2014a) state that climate change represents the most significant problem of today, which is characterized by increased temperatures, unbalanced amounts of precipitation, as well as the increasing presence of extreme weather events.

Accordingly, the Report of the Intergovernmental Panel on Climate Change (IPCC 2014a) indicates that there are already moderate risks of future extreme weather events, and that with further warming, these risks would become very high and that each of the last 3 decades on the Earth's surface has been warmer than any previous decade since 1850. The period 1983-2012 was probably the warmest 30-year period in the last 1400 years in the northern hemisphere. According to all scenarios, the air temperature will rise during the 21st century, and it is very likely that heat waves will occur more often and last longer, and that extreme climate events when it comes to precipitation will become more intense and frequent in many regions.

As a result, forest management measures would have to adapt to changing environmental conditions - new knowledge and strategies in forest management would be needed; the formation of new climate zones would affect the formation of new forest ecosystems (significant changes in forest composition), it will be necessary to define new areas of species distribution, etc.

Lindner *et al.* (2010) states that, due to climate change, production in forests in the area with moderate-continental climates is likely to decrease in areas subject to water stress and increase where increased water evaporation due to elevated temperatures is balanced by increased rainfall. In addition, the effects of climate change on certain species can be positive or negative depending on habitat conditions and regional climate change, and it is especially emphasized that the beech will face serious problems due to the increase in temperatures.

ICP Forests Programme (International Cooperative Programme on Forest Condition Monitoring) has been continuously performed in the Republic of Serbia since 2003. Within Level I of this program, a network of approximately 6000 sample plots (bioindication points) has been established in Europe to monitor the health status of forests and their spatial and temporal changes on a broad basis during the necessary period. Within Level II, intensive monitoring of the impact of transboundary air pollution on forest ecosystems is performed at over 800 bioindication points (NFC, 2017, 2018).

Current climate change

Current climate changes are manifested in the change of basic climate elements. The increase in the mean global temperature compared to the value from the pre-industrial period reached a value of 1°C (IPCC, 2013). The main cause of global warming of the "modern age", i.e., period since the industrial revolution, is the increase in the concentration of greenhouse gases in the atmosphere, and the most responsible is carbon dioxide. A warmer atmosphere can also contain a larger amount of water vapor, which is also a greenhouse gas, and that is one of the examples of positive feedback effects of increasing average global temperature which further enhances the greenhouse effect (Popović and Vuković, 2019).

Due to the extreme complexity of the climate system and the complex interactions between its components, climate models can be used to assess the sensitivity of the climate system to changes in greenhouse gas concentrations, which can simulate climate change and changes within the Earth-atmosphere system with sufficient accuracy (Vuković, 2018; Vuković *et al.*, 2018). To analyze the reliability of future climate projections, the results of several climate models, the so-called model ensembles, are used. Global analyzes of the observed data and data obtained by simulations of climate models unequivocally indicate the fact that the cause of climate change over the last century is increased greenhouse gas emissions which are a consequence of the development of industry, transport and economy, and that is the reason why these emissions are called "anthropogenic emissions", and why the "anthropogenic impact" has been identified as the main cause of climate change.

According to the Fifth Report (IPCC, 2013), based on the results of the global distribution of changes in mean annual temperatures and precipitation amounts for the period 2081-2100 compared to 1986-2005 according to *Representative Concentration Pathways* (RCP), the largest temperature increase is expected in the northern hemisphere above continental areas (over 2°C according to RCP4.5 and over 4°C according to RCP8.5), and especially in the northern regions (over 3°C according to RCP4.5 and over 5°C according to RCP8.5). The global distribution of changes in the mean annual amount of precipitation is more complex. In the Mediterranean area, the results indicate a reduction in annual rainfall by 10-20% according to RCP4.5, and even over 20% according to RCP8.5. Moving to the north, precipitation changes change sign and projections indicate an increase in precipitation. The

transition area, which includes the Western Balkans, shows increased uncertainty in the model results, which requires more detailed analysis using regional models with higher spatial resolution.
The anthropogenic impact causes a relatively sudden disturbance of the climate system, which causes about 10 times faster climate change than any natural factor (Diffenbaugh and Field, 2013).

Climate change in the region

A study conducted for the Western Balkans (Vuković and Vujadinović Mandić, 2018), in accordance with the scenarios of greenhouse gas changes and the analyzed climatic periods of the IPCC Fifth Report (IPCC, 2013), using regional high-resolution models, shows that the rise in temperature in this region is happening faster than the rise in mean global temperature in general. The change in mean temperature, obtained for the period 1986-2015 compared to the period 1961-1990 is 1.2°C, which is the reason why this region is considered as one of the areas most endangered by climate change in the world. The largest changes in seasonal climatic values of temperature and precipitation were observed during the summer season. The increase in the average summer temperature is the largest in the central part of the region, especially in most of Bosnia and Herzegovina, and exceeds 2°C, while the amount of precipitation is reduced by 10-20%. The average annual amount of precipitation in most of the region increased by 5-10%. The northern parts of the Republic of Srpska are slightly less endangered by the summer deficit of precipitation than the extreme southern regions. In the southern regions, the reduction of precipitation during the summer is over 20%. The frequency of extreme weather events, such as heat waves, droughts, extreme rainfall and floods, has also been observed in the Western Balkans region.

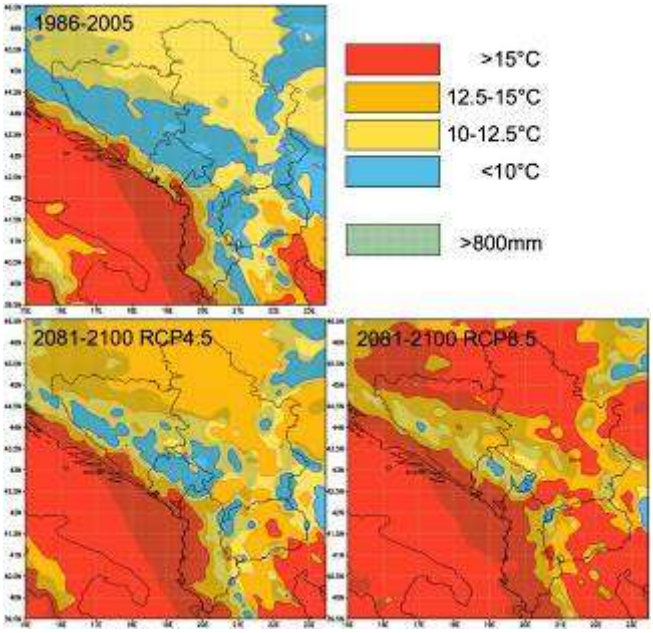


Figure 1 The mean annual temperature and the sum of precipitation for the reference period 1986-2005 and future period 2081-2100 according to RCP4.5 and RCP8.5 scenarios, obtained as the median of the ensemble of nine regional models (Babić, *et al.*, 2018; Vuković and Vujadinović Mandić, 2018).

Future climate change in the Western Balkans provides an estimate of the range of the most likely future climate conditions by the end of the 21st century (IPCC, 2013): the results

obtained according to the RCP4.5 scenario indicate the lower limit of changes and the results RCP8.5 indicate the upper limit of changes. According to the RCP4.5 scenario, an additional temperature increase of 1.7°C is expected by the end of the century, and 4.0°C according to the RCP8.5 scenario. This indicates that the total temperature increase in the region will be probably in the range from 2.9°C to 5.2°C compared to the 1961-1990 reference period. Figure 1 shows the regional distribution of mean annual temperature values for the reference period 1986-2005 and the period 2081-2100 according to scenarios RCP4.5 and RCP8.5. Reducing the prevalence of areas with colder temperatures, i.e., with an average annual temperature below 10°C, characteristic for the habitats of current forest species, decreases drastically according to the RCP4.5 scenario, while according to the RCP8.5 scenario it almost completely disappears (Babić *et al.*, 2018; Vuković and Vujadinović Mandić, 2018). The estimated speed of isotherm moving in the region is about 1000 km to the north and about 1000 m to higher altitudes in 100 years.

Characteristics of climate change in the Republic of Srpska

Data from meteorological measurements from the following climatological stations were used to show the climatic conditions of the Republic of Srpska: Banjaluka, Prijedor, Doboj, Bijeljina, Sokolac, Bileća, Trebinje for two periods: reference period 1981-2010 and the five years 2014-2018. The annual and average monthly values of the most important climatic elements are presented: temperature conditions and pluviometric regime. Climate type affiliation was determined by the method of Thornthwaite (Im), Lang (KF) and Ellenberg (EQ). The procedure of work according to (Babić and Unkašević, 2019) was applied.

The average annual air temperature for the period 1981-2010 ranges from 7.0˚C for Sokolac to 14.4˚C for Trebinje. Banja Luka, Prijedor, Doboj and Bijeljina have a uniform average annual air temperature from 11.1 to 11.7°C, while the area of Bileća and Trebinje represents a slightly warmer part of Republika Srpska with a higher average annual temperature, which is the influence of the Mediterranean. The average annual air temperature for the period 2014-2018 ranges from 8.3°C for Sokolac to 15.3°C for Trebinje, which is an average increase of 1.1°C compared to the reference period for the entire territory of Republic of Srpska (Figure 2). The smallest increase in the average annual air temperature was recorded for Bileća (0.6°C), and the largest for Bijeljina (1.5°C). The warmest month is July with an average temperature of 25.7°C for Trebinje, which is an increase in temperature by 1.4°C. The average winter temperature ranges from -0.7°C for Sokolac to 6.9°C for Trebinje, which is an increase in temperature in the winter season in the entire area of Republika Srpska by 1.3°C.

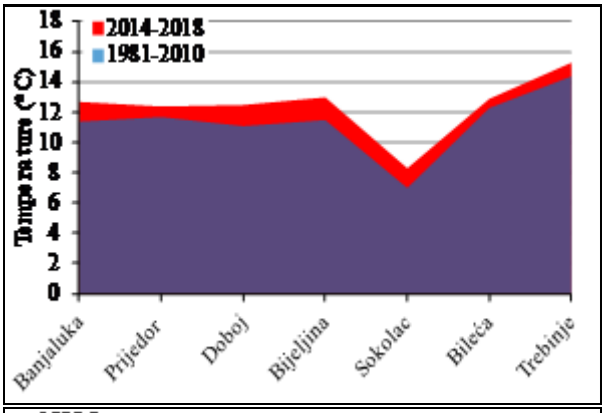


Figure 2 Comparative characteristics of the temperature regime (annual values)

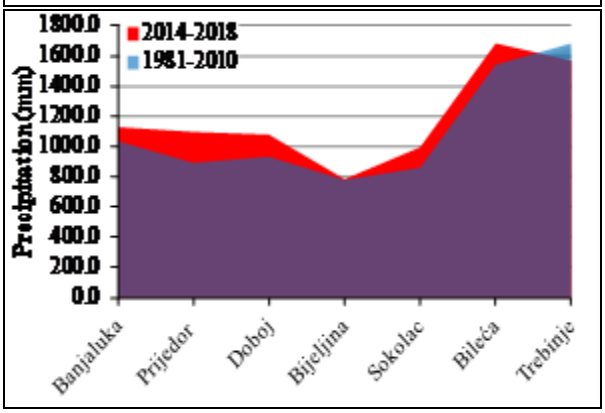


Figure 3 Comparative characteristics of the precipitation regime (annual values)

The lowest annual amount of precipitation for the period 1981-2010 is in the area of Bijeljina (778 mm), and the largest in Trebinje (1691 mm). Prijedor, Doboj, Bijeljina and Sokolac have below 1000 mm of precipitation per year, Banja Luka has over 1000 mm, and the area of eastern Herzegovina (Bileća and Trebinje) has over 1500 mm. There is no typical continental type of pluviometric regime in the territory of the Republic of Srpska. The average annual amount of precipitation during the year for the area of Banja Luka, Prijedor, Doboj, Bijeljina and Sokolac is with one pronounced maximum in June, from 94 mm for Sokolac to 117 mm for Doboj, and with one minimum in February, from 44 mm for Bijeljina to 59 mm for Banja Luka. The average annual amount of precipitation during the year in the area of Eastern Herzegovina is with one pronounced maximum in November with an average of 220 mm for Bileća and 252 mm for Trebinje and with one minimum in July with an average of 48 mm for Bileća and 43 mm for Trebinje. The average annual amount of precipitation for the period 2014-2018 ranges from 785 mm for Bijeljina to 1684 mm for Bileća. Except for the Trebinje station, there was an increase in the amount of precipitation at an annual level in relation to the reference period in the entire area of the Republic of Srpska. The largest increase in the annual amount of precipitation was recorded for Prijedor 188 mm, and the smallest for Bijeljina 7 mm. A significant decrease in precipitation of 110 mm was recorded for Trebinje (Figure 3).

For the needs of research in biological and biotechnical sciences, especially in the field of forestry, climate indices and classifications are used, which indicate the influence of basic climatic elements on the entire vegetation and its vertical and horizontal distribution (Babić and Unkašević, 2019).

Climate classifications of the area of Republic of Srpska for the two studied periods (reference period 1981-2010 and the period 2014-2018) show the following:

Lang's bioclimatic classification for the reference period indicates that the analyzed area of Prijedor and Bijeljina is characterized by a *semi-humid climate*. The areas of Banja Luka, Doboj, Sokolac, Bileća and Trebinje are located in the area that represents *a distinctly forest area* with a *humid climate*. In the period 2014-2018, the entire territory of the Republic of Srpska belonged to the *distinctly forest area* with a *humid climate*, except the area of Bijeljina where the *semi-humid climate* prevails.

According to Thornthwaite's annual climate index (Im) for the reference period 1981-2010, in the area of Bijeljina, as the driest part of the Republic of Srpska, the *subhumid-humid climate* of type C_2 dominates, in the area of Prijedor and Doboj the *slightly humid climate* of type B_1 dominates, Banja Luka and Sokolac have a *moderate humid climate* of type B_2, and the area of eastern Herzegovina *perhumid climate* of type A. In the period 2014-2018 in most of the Republic of Srpska, the climate index increased, except for Bijeljina and Trebinje, where it decreased. The climate in the area of Bijeljina has not changed. The northwestern and central part of the Republic of Srpska (Banja Luka, Prijedor and Doboj) have a *moderately humid climate* of type B_2, the area of Sokolac has an *intensified humid climate* of type B_3, Trebinje has a *strongly humid climate* of type B_4, and the area of Bileća is dominated by *perhumid climate* of type A.

The impact of climate change on forest ecosystems - current knowledge about future impacts

Many authors indicate that the rise in temperatures in recent decades have caused an increase in drought stress to many tree species (Bernhofer *et al.*, 2009; Adams *et al.*, 2011; Gillner *et al.*, 2013). As a consequence of climate change, forests may face intense changes in terms of time, intensity, frequency and extent of disturbances (Dale *et al.*, 2001). There is a high probability that changes in temperatures and precipitation will have a strong direct impact on forests. Some vegetation models predict that forest vegetation will move to areas with more favorable climatic conditions, which will eventually result in forest expansion and replacement of up to 50% of the existing tundra area (Kirilenko and Sedjo, 2007). According to Ducić *et al.* (2011) climate change, among other things, has contributed to changes in the altitude of the upper limit of trees in the high mountain area. Climate change and its potential impact on forest ecosystems have been studied by Krstić *et al.* (2012) in Serbia, Trbić *et al.* (2012) in the Republic of Srpska, and others.

Forestry has a long tradition of adapting practices to changing, ecological, economic and social conditions, where also global changes (climate change, the presence of invasive species, etc.) are no exception (Puettmann, 2011).

Krstić (1999) states that the representation and distribution of certain types of forest vegetation are mostly adapted to the existing climatic conditions. The assumed climate changes will certainly affect the forest, and this especially refers to the predicted temperature changes (high temperatures during the summer, warmer winters, more frequent spring and autumn frosts), changes in precipitation (frequency and duration of drought), stronger winds. etc.

Based on the Report of Working Group 2 about climate change (IPCC WG2, 1990), according to Krstić *et al.*, (2010) global and regional climate change, among other things, would be manifested as follows:

- the increase in immission would be doubled, and ecological conditions would change, which would result in physiological and biological changes of the living world;
- forests would be difficult to adapt and their decay would increase;

- the survival of many species of flora and fauna would be endangered, especially in the most sensitive areas (semiarid) where many species are "closed" in their biological limit;
- relatively small climate changes would create a major problem with water resources in many areas.

The following ascertainments are stated in the Report of the Second Working Group on climate change (IPCC WG2, 2007) (www.hidmet.gov.rs/podaci/meteorologija, 2010):

A. Many natural systems are affected by regional climate change, especially with the rise in air temperature due to global warming: moving of zones with certain species of plants and animals towards the north and higher altitudes; from the early '80s until now there is a trend of earlier leafing of vegetation and increasing the length of the growing season; significant changes in many physical and biological systems - more than 89% is consistent with the direction of expected changes in response to global warming.
B. The capacity of many ecosystems to adapt naturally to climate change will be overcome during this century by a combination of climate change accompanied by disturbances (such as floods, droughts, fires, insects, ocean acidification), and other global change factors (land use change, pollution). On average, 20-30% of plant and animal species will disappear if the temperature increase is greater than the range of 1.5-2.5°C with the corresponding increase in CO_2 concentration.

The role of forestry in the context of climate change

An important place of the forestry sector is in the adaptation to climate change, mostly due to the impact of forests on reducing temperature and maintaining humidity. The importance of preserving existing forest systems and expanding their global distribution was recognized in an extensive IPCC study "Climate change and Land" (IPCC, 2019), and the indirect impact on preserving microclimatic conditions and mitigating the increased effects of drought in "Land- Drought nexus" (Reichuber *et al.*, 2019).
Forest ecosystems are extremely sensitive to climate change. Natural forest migrations are much slower than the movement of optimal climatic conditions towards higher altitudes or latitudes, so the danger of their extinction is extremely high. For this reason, it is necessary to plan adaptation measures and their application in forestry in order to preserve forest areas and the sustainability of industries that rely on forestry.
According to Bernier and Schoene (2009), there are three possible approaches for adapting forests to climate change: no intervention, reactive adaptation and planned adaptation. According to Spathelf *et al.* (2015) the use of species of different provenances and exotics species, or assisted migration of well-adapted tree species from other climatic conditions can improve the adaptive capacity of forests. Also, the regeneration of stress-resistant pioneer species can be supported by the application of different silvicultural systems. Krstić *et al.* (2010) indicate the tasks and necessity of applying appropriate silvicultural measures in forestry in accordance with climate change in the forest.
The importance of the fight against climate change in forestry is also contributed by the fact that forest ecosystems are significantly inert, where the initiated changes are harder to stop and the consequences of negative impacts cover a period of one century.
Climatic parameters, the observed vulnerability of forest ecosystems, and measures of adaptation to climate change are as follows:

- **Climatic parameters:** temperature rise, change of precipitation regime, droughts, intensification of precipitation, heat waves;

- **Vulnerability:** rapid change in the distribution of climatically favorable areas, increased vulnerability to diseases and pests, tree growth and development disturbance, increased fire risk, insufficient water availability, flood damage;
- **Adaptation measures:** a selection of species and populations more tolerant to changed climatic conditions, promotion of mixed forests, the adaptation of forest management, implementation of fire and flood protection measures, increase groundwater levels in dry periods, afforestation as a measure against erosion, raising awareness of the importance of forestry in combating climate change, training on monitoring the endangerment of forest ecosystems and implementation of adaptation measures.

Examples of practical measures for adapting to climate change and mitigating their impact in relation to forestry according to Murthy *et al*. (2011) are: anticipatory planting of species along latitude and altitude, promote assisted natural regeneration, promote mixed species forestry, promote species mix adapted to different temperature tolerance regimes, develop and implement fire protection and management practices, adopt thinning, sanitation and other silvicultural practices, promote in situ and ex situ conservation of genetic diversity, develop drought and pest resistance in commercial tree species, develop and adopt sustainable forest management practices, expand protected areas and link them wherever possible to promote migration of species, conserve forests and reduce forest fragmentation to enable species migration, adoption of energy efficient fuelwood cooking devices to reduce pressure on forests.

Conclusions

Regional climate models for the Western Balkans show that the rise in temperature in this region is faster than the rise in average global temperature in general. The change in mean temperature, obtained for the period 1986-2015 compared to the period 1961-1980 is 1.2°C. The increase in the average summer temperature is the largest in the central part of the region, especially in most of Bosnia and Herzegovina, and exceeds 2°C, while the amount of precipitation is reduced by 10-20%.

As a consequence of climate change, forests may face intense changes, because there is a high probability that changes in temperatures and precipitation will have a strong direct impact on forests. Some vegetation models predict that forest vegetation will move to areas with more favorable climatic conditions - a moving of zones with certain species of plants and animals towards the north and higher altitudes.

Forestry has a long tradition of adapting practices to changing environmental conditions, and in the context of climate change, forest management approaches are especially proposed to mitigate climate change. There are three possible approaches for adapting forests to climate change: no intervention, reactive adaptation, and planned adaptation. The necessity of applying appropriate silvicultural measures in forestry is especially important, having in mind the impact of climate change on the forest - assisted natural regeneration, promotion of mixed forests adapted to different temperature tolerance regimes, forest thinning, application of sanitary and other types of silvicultural measures, conservation of genetic diversity (biodiversity), adoption of sustainable forest management practices.

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TYPES OF FORESTS AND TYPOLOGICAL BASIS OF FORESTS IN MULTIFUNCTIONAL PLANNING AND FUNCTIONAL SUSTAINABILITY OF THE ENVIRONMENT

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Abstract

The basis of the typological study of forests is based on the study of forests as a complex biogeocenosis, variable in space and time. This implies division and making of a classification on a basis that such parts represent separate and recognisable wholes arising from the biogeocenotic study and classification, which relates to the ecological-coenological segment of the forest and, potentially-productional segment and aspect of forest and forest as a whole. In this sense, natural forest ecosystems are formed, established and classified in relation to the studied characteristics - as forest types. The methodology itself, and in that sense the methodological approach, provides the starting point in planning orientation and planning, which are related to different and multifunctional approach and preservation of natural ecosystems, natural sustainability and functional sustainability of natural ecosystems and environmental protection as a whole, multifunctional forest functions in the overall environmental sustainability. It is especially important to establish a link between forest classification, in that sense - forest types, and prominent functional requirements in planning, provision and optimisation of a specific forest function, for example, establishing a link between the typological basis in functional forest planning and their planning orientation when it comes to the specific function of forests. In particular, the anti-immission function of forests is expressed through biologically functional requirements and other bio-technical and specific requirements that specifically relate to the biogeocenotic approach to the study of forests and on that basis its classification into forest types. The basic starting point and structural orientation of this research is to consider the possibility of improving the overall potential of forests and their multifunctional sustainability in multi-purpose planning and functional sustainability, environmental protection and especially the relationship of primary structure of forest types to climate change in that sense, through biological, biotechnical, technical and other specific requirements in order to create a functional optimum of a specific forest and planned zoning in specific conditions and wider social needs.

Keywords: *forest type, typological basis, forest functions, functional requirements, planned zoning.*

Introduction

The oldest records on the perception of forest resources and potentials referred to the forest and forest areas as an unused area, that is, to the potential opportunities and potential of agriculture. Consequently, man's influence has existed from the very beginning of his settlement in a certain territory. Human influence grew with the growth of technological development, urbanisation and industrialisation. The consequence is the lack of a broader foundation on the importance of forests in entire social potentials, and consequently, a broader and unplanned point of view in terms of deforestation on large areas in favour, in earlier stages primarily, of agricultural land and later land for other purposes. Consequently, man lowered the upper limit, and raised the lower limit of the widespread forest. However, in the era of intensification of these processes, we are getting the recognition and evaluation of

forests as a significant factor in the overall sustainability of natural and structural resources of the biosphere and the overall sustainability of life processes and the environment as a whole.
Beneficial effects of forests (Esten and Reder, 2001; Medarević, 2006), are expressed through water quality, water quantity, flood protection, soil quality and fertility, erosion, landslides, landslide protection, air conditioning; local and regional, global, noise protection, dust protection, absorption of harmful substances, nitrogen circulation, cultural and social aspects; natural monuments, historical forms of forest use, rest and recreation, aesthetics, nature protection; species protection, biotope protection, process protection, protection of regulatory functions of ecological systems and economic and social aspects; wood, other forest products (fruits, mushrooms, game, etc.), income from labor, income from forest ownership, social structure, spatial structure (settlement structure, rural space), security of supply (economic aspect).
Forest valuation in modern planning concepts is initially related to multipurpose use, expressed through the evaluation of all aspects of forests and the importance for the overall potential and coherence of planning and planning development. This refers to the value elements and their assessment and evaluation related to the planned zoning and functional attachment to a specific forest function. Multipurpose use in itself is not unified, but is more or less highlighted through different levels of planning and evaluation of different aspects related to specific and concrete needs. This results in different forest functions. Multiple positive effects and multipurpose use of forests are not achieved by themselves, but it is necessary to create a basis through planning concepts (modern forest management planning), in terms of defining a management system (multifunctional management), which will ensure multipurpose and permanent use of forests (functional durability). Such a concept requires, as is only achievable, with a different approach at the outset. This implies the creation of a realistic basis, which is based on the study and characterisation (classification) of the forest as a complex biogeocenosis (Milošević, 2012).
The methodological procedure and setting of biogeocenotic study and classification of forest - typological definition of forest - in the very basis and finally through the classification of typological structure, gives the initial elements of evaluation as well as their assessment and assessment related to a specific forest function. The type of forest through the methodological procedure and different methodology gives a certain possibility of assessing and estimating the value of individual functions and value elements that are the starting point in providing ecological and coenological functional durability.
For example, the production function - potential and existing productivity in the basic-initial phase related to that segment of a specific type, part of the forest - section in practical planning. *Protective functions* - primary edificatory structure in relation to the existing, ecological-coenological relationship of a specific species or species on a specific forest type, especially when it comes to artificially raised stands on different forest types with priority different purposeful provision of different social needs.
Considering the methodology of typological definition and classification of forests on the one hand and the possibility of applying the initial elements expressed through the type of forest on the other hand, in multifunctional planning and multifunctional provision of permanent and optimal forest use, the goal of this aspect and this paper forest functions and overall multifunctional forest effects.

Basic typological - methodological settings of typological definition of forests

Modern planning and planned zoning of forests arises from multifunctional social needs with the ultimate goal of providing a functional optimum of a particular forest and forests as a whole, on the one hand, and thus the preservation of the biodiversity structure and the

different and overall contents of the total potentials of the environment and natural resources. Consequently, it is necessary to create a starting point for realistic, multifunctional planning of forest functions, defining forest parts and forests for a specific purpose in line with priority and specific needs in certain zones of different structure.

That is why it is necessary to investigate, to study a specific forest or forests as a whole on a biogeocenotic basis, namely, to typologically define and classify the forest when it comes to the basic - specific purpose, or forests, when it comes to the global purpose of forest complexes.

The significance of this derives from the methodological procedure of typological definition and classification of forests (Milošević, 2006; 2012), which is based on direct research and studies. This implies the research of the ecological-cenological aspect and ecological-cenological classification, that is, the research of the synergistic relationship of ecological factors with the primary dynamic relations and the research of the primary vegetation and the primary edaphotope. On that basis, the classification or ecological-coenological structure is defined, which represents the basic starting point for a complete typological definition of forests - *forest types*. This refers to the fact that for each defined ecological-coenological whole - ecological unit - potential productivity is established and researched. This completely rounds the typological definition of forests on the one hand, and provides the basic elements for further planning, which are related to different biological and other requirements related to different forest functions.

Different functions of planning and zoning have different and specific structural requirements (Medarević, 1991).

From the aforementioned, we can conclude that the definition of the basic classification unit in the typology of forests is - *forest type*.

Forest type is a segment of a forest complex or a forest as a whole, which consists of a large number of parts that are characterised by homogeneous or approximately homogeneous ecological and vegetation composition and the achieved level of potential production and its development, stands of autochthonous tree species within which a special (in relation to other types) planning procedure harmonised with the priority purpose of stands is applied (Milošević, 2012).

Forest type includes parts of forests with equal ecological and potential developmental and production characteristics of stands of autochthonous tree species to which a specific management treatment corresponds (Jović et al., 1976).

Analysis of typological basis in functional requirements and multifunctional planning

The basic starting point of multifunctional planning and multifunctional needs of society in general, is to ensure the optimal state of the specific function of the forest in specific conditions with specific social needs.

Consequently, the optimal condition of the forest related to a certain purpose, is the condition that best ensures functional sustainability and fulfilment, and expressed through various planning requirements.

The evaluation of forest ecosystems in modern planned zoning is not only related to the production function, but at the same time and equivalently through other forest functions.

The forest, as the most perfect form of nature's historical activity, represents the most important natural basis of life. The value of forest ecosystems nowadays is not related to the quantity and quality of wood volume, but the value of the forest as the most important object for oxygen production, protection of the terrain from erosion, existence of water sources, microclimatic effects, health effects, etc. (Milošević, 2012).

The prominent and scientifically based multifunctional significance of forests requires the need to define a specific forest with a certain function.
Consequently, in the previous plans and planned zoning of forests, the basic initial and immediate functions of forests are preventively related to the preservation of total biosphere resources and the overall environment.

- forests and forest habitats with production functions - for forest complexes for which no special purpose has been determined by special legal acts;
- forests and forest habitats with production and protection functions - for forest complexes for which a special purpose has been determined by special legal acts, and at the same time the maximum production and use of production potentials of habitats are not in conflict with any other general management goal;
- forests with priority protection functions - for forest complexes whose priority management goal is related to the protective role of the forest (water source areas, erosion-labile areas, etc.);
- forests intended for recreation and general cultural and educational functions - for forest complexes where social interest is a priority, e.g. use of space for recreation or educational teaching process (Ada Ciganlija for recreation, Mt. Goč as an educational teaching centre, etc.);
- mostly include purposes determined by the law on environmental protection (or other laws) for specific complexes and the possibility of a proposal in the basis of management for declaring a specific forest is not excluded for a forest park, animal park, reserve, etc. (Banković, Medarević, 2003).

Typological basis and functional requirements of anti-erosion function of forest

In order to fully provide and functionally maintain this forest function, it is necessary to establish and consider the basic functional requirements expressed through levels of planning through planned zoning of stands. According to Medarević (Medarević, 1991), these requirements are: biological, bio-technical, technical and specific functional requirements.
The typological basis in ensuring the optimal-functional condition expressed through the planning basis and planned zoning of stands in this function, is expressed through consideration and assessment of the existing condition in relation to the primary ecological-coenological potential and assessments of production effects of the existing condition, selection of tree species for forestation on a typological basis and dynamics of planned procedures based on the current condition in relation to the primary structure of forests types or forest type.
In the assessment of the existing condition, which is very prominent through the provision of optimal condition, especially when it comes to this planned zoning of forests, is the current assessment of ecological-coenological adaptability and vitality of different types of allochthonous and autochthonous character on the one hand, and consideration on that basis of further planning-functional sustainability.
This is especially stated and researched when it comes to both indigenous and non-indigenous tree species in the habitats of different types of forests (Milošević, 2011; 2016, Bićanin and Milošević, 2015a, 2015b; Milošević, 2017a, 2017b; Novaković et al., 2016; Milošević, 2019a, 2019b).

Typological basis and functional requirements of water protection function of forest

This function of forests is of multiple importance, and primarily it serves for the sustainability and perseverance of the purity of standing, ground and running water. There is a special

emphasis on these planned zoned forests in the permanent quality assurance and permanent release of water, which is further reflected in the starting point of the overall potential and sustainability of environmental quality.
When it comes to the planned evaluation of functional elements, they are multiple.
When it comes to typological research as one of the initial value elements that refers primarily to the biological aspect of functional requirements, and according to Medarević (Medarević, 1991), from the aspect of the application of the forest type, it is a choice and planned forcing and planned orientation in that sense, a tree species on a typological basis.

Typological basis and functional requirements anti-immission function of forests

It has been indisputably proven that forests have a broader aspect of positive effects on reducing the negative effects of air pollution, starting from different agents of different origins and compounds in that sense, all the way to reducing the level of traffic noise which in synergy with different pollutants leaves degradation and directly affects the environment, that is, it reduces the potential for quality and the natural balance of the environment.
A special aspect in the consideration and the possibility of combating the negative effects on the entire component of the environment is the aspect of the impact of forests and the possibilities in that sense of the sustainability of the quality of the environment.
This primarily refers to biological functional requirements, and especially the typological segment in comprehensive functional planning, the choice of tree species is harmonised with the bioecology of a particular species in specific habitat conditions - habitat conditions of the forest type with one, and the rank of priority according to the immission resistance of a certain species.

Typological basis and functional requirements of climate protection function of forest

The typological aspect of planned zoning is highlighted by multiple needs and multiple aspects of balancing and reducing the extremes of climatic factors and creating climatic conditions in that sense with as few extreme exposures of a particular object.
In planned and normal, comprehensive planning, the typological aspect refers to the selection of tree species adapted to specific habitat conditions and specific protection needs. Permanent structural protection is based on the initial basis for the selection of tree species appropriate to the typological basis and further to the planning and cultivation modalities.

Typological basis and functional requirements of road protection function of forest

Road protecting forest implies protection from the harmful effects of gases and noise, and at the same time reflects the stability of roads. By defining the function and action of these forests in this way, the priorities in protection are clearly highlighted. (Medarević, 1991).
Namely, the research of this aspect of forests has indisputably confirmed the significant impact of forests and different types of forests on the reduction of traffic noise levels (Milošević, 1996, Milošević, 2016).
Functionally sustainable requirements, when it comes to dedicated zoning of forests in this function, are highlighted through biologically functional requirements. These requirements are also related to the selection of tree species adapted to specific typological conditions of habitats as a starting point for the optimisation of specific management-planning procedures, and they concern the origin, structure, structural form, dynamism of planning works, etc.

Typological basis and functional requirements of production function of forest

Evaluation of forests and forest ecosystems and planning orientation was related and expressed to the scientific foundation and importance of other, primarily ecological forest functions, that is equivalence of production and ecological different forest functions.
The production function of the forest was first established as cognition of the beneficial effects of the forest. Therefore, the previous evaluations (recording of the condition of forests) were mainly related to this function, independently, to a greater or lesser extent, of the established multiple effects of forests (Medarević, 1991). However, considering the broader economic aspect, the purposeful zoning and valuation of these forests is still the most pronounced. In this sense, multiple planning concepts have been built to ensure the ultimate economic effect. The initial basis for different modalities within this forest function is mainly and primarily based on the typological basis of planning. This applies to the following: *selection of management objectives, determination of the purpose of forest complexes and parts of complexes, formation of management classes, determination of optimal mixture size, selection of optimal structural shape, felling cycle (determined duration of production process), definition of optimal state of forest type harmonised with priority purpose (functional optimum), evaluation of the existing condition in relation to the optimal condition of the forest type* (Milošević, 2012).

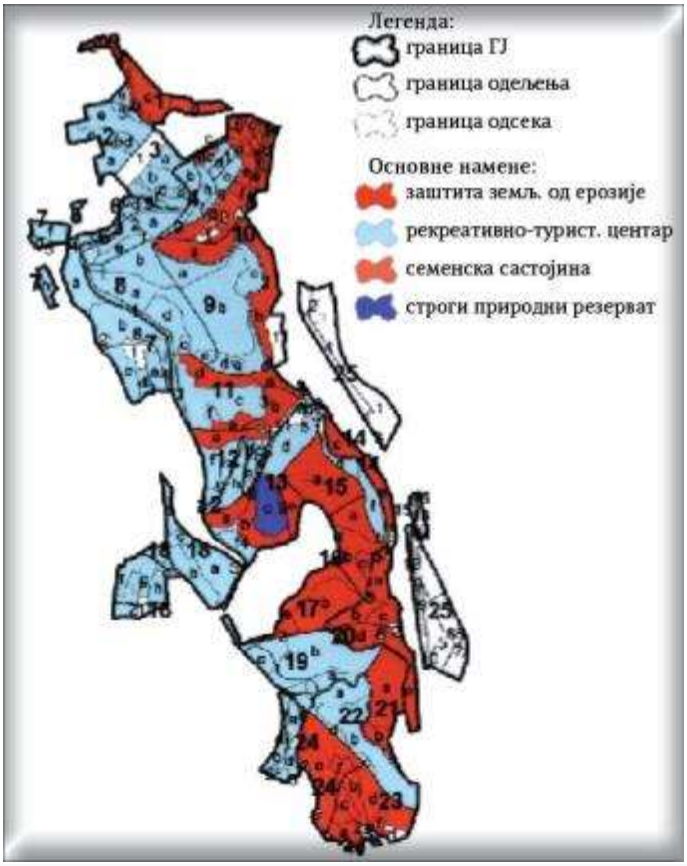


Figure 1. Dedicated zoning of forests in park forest "Košutnjak"

Typological basis and functional requirement of recreational function of forest

This function of forest, that is, planned zoning of forests in providing optimal content is of a specific character. This primarily refers to the structural content of the overall environment, and consequently the planned action to achieve and ensure that goal. This implies the

construction and diversity in the vegetation-ambient and functional structure and the participation of as many indigenous and non-indigenous tree species as possible. Consequently, according to Medarević, (Medarević, 1991), biological-functional requirements also arise and refer to: primarily the choice of tree species on a typological basis, forcing heterogeneous structural forms such as selective forest, group-diverse forest, two-storey stands and stands with reserves, formation of mixed stands with equal treatment of trees, shrubs and herbaceous plants.

In functional sustainability and optimisation of overall contents in that sense, when this forest function is in question, it is of special importance to research and define the ecological-coenological vitality of tree species that are in secondary habitats, that is, habitats of other forest types. The research of ecological-coenological relationship and ecological sustainability and vitality is directly related to the optimisation and provision of the overall functional requirements of this forest function. Ecological-coenological vitality, especially of tree species in secondary habitats, is achieved by direct research and typological classification of specific habitat conditions into forest types (Milošević, 2011, Bićanin, Milošević, 2015, Bićanin, Milošević,2015, Milošević, Novaković Vuković, 2017, Milošević, Novaković-Vuković 2017, Novaković-Vuković, Milošević, 2016, Milošević, Novaković-Vuković, 2019, Milošević, et all. 2019, Milošević 2016).

Types of forest in environmental protection

The condition of forests directly reflects on the condition and sustainable balance of basic living resources and the environment as a whole.

This importance of forests stems from the complex relationships and ecological processes that make up the form of a forest ecosystem and, accordingly, the formation of various dynamic and structural forms. In this respect, the type of forest represents a specifically marked and separated natural whole. Consequently, in practical measures and works on the preservation and improvement of basic resources and environmental quality, a typologically defined forest and a specific type provides a realistic and reliable basis for planning projections and measures in given natural conditions.

In the planned measures in the protection of the natural environment, the practical application of the typological basis and type of forest refers to:

- realistic determination of the basic purpose adapted to different types of protection of the basic components of living space and multipurpose use of forests;
- structure, characteristics, protection and sustainability of biodiversity;
- protection of ambient units;
- protection soil from erosion and other forms of soil protection;
- protection of watercourses and water supply;
- climate protection;
- biotope protection;
- lifting
- seat belts (immission protection, noise protection, field protection belts, etc.) (Milošević, 2012).

Biodiversity and forest type

The FAO definition of forest biodiversity reads: "…variability between living organisms in the forest and the ecological processes of which they are a part, this includes diversity in the forest within species, between species, between species and ecosystems and landscapes" (1992).

From this definition of biodiversity, it is clear that the typological (ecosystem) diversity and diversity of a complex constitutes the biodiversity of that complex.
Each type of forest represents one forest ecosystem with its own characteristics in terms of composition, structure, productivity, dynamic processes, etc. Each forest ecosystem is characterised by its shape and composition of species, horizontal and vertical structure, mutual actions and coactions and the characteristic dynamism of ecological processes, which overall represents, among other things, the biodiversity of a complex. Knowledge of the typological composition of a complex, in addition to simultaneously knowing the characteristics and structure of biodiversity, forms the basis for preserving the primary structure, sustainability and durability (Milošević, 2012).

Conclusion

Considering local, regional and global trends and structural dynamics and functional-economic orientation more broadly, forest ecosystems and forests as a whole are becoming the primary object of sustainable development and overall potentials and sustainability of the quality of the environment as a whole.
Consequently, the need for different functional public benefit contents of forests for the society has changed and forests can already be classified according to the planned priority into protective, social, primarily, recreation and production-protective.
In order to fully respond to the public and emerging social needs for the viability of total resources and natural potentials, a more pronounced and well-founded environmental aspect in planning is needed.
That means, a planned approach in providing public forest functions based on planned functional-sustainable requirements of a specific forest function.
This approach is realistically achievable and is a planned priority in the study and classification of forests on ecological-typological grounds.
Biological-functional requirements are also different for different functional contents and optimisation of a specific function.
In all of the above functions and the analysed functions, the biological functional requirements stand out as the starting point in functional security. With what it is, this aspect from function to function more pronounced or less pronounced.
In that sense, all protective functions and social - recreation, are based and planned exclusively related to that aspect and ecological-typological bases of planning.
This is also expressed in the production-protective functions, and is expressed on the one hand through the stability of achievable production and the vitality of edificators and in that sense, and development of planning modalities within defined forest types and typological classification on the other hand.

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IMPORTANCE OF TESTING THE DYNAMIC MODULE OF DEFORMATION ON FOREST ROADS

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Abstract

Intensive development of forest road infrastructure through the construction of new and reconstruction of existing forest roads, requires fast and quality methods for assessing the quality of performed works. In order to ensure the long-term functioning of the forest road, its bedrock and aggregates must provide a solid and stable base. During the construction of forest roads, it is necessary to control the performed works constantly in order to ensure that the road construction materials are sufficiently compacted to ensure this condition. The road construction of the forest road must be able to withstand the loads arising from traffic. This bearing capacity is often influenced by the degree of compaction, the moisture content and the type of soil on which the road is built. The practice of examination of the degree of compaction has been present for a long time in our civil engineering, but apart from individual cases, it has not found wider application in the construction of forest roads. The paper presents the importance of testing the dynamic modulus of deformation on forest roads and gives an overview of the research conducted so far using a dynamic plate with a falling weight. The research was conducted on four forest roads in different parts of Serbia, whose road construction was built of stone aggregate of shale, peridotite and limestone. Testing of the dynamic deformation modulus was performed with a dynamic plate of the brand ZORN type ZFG 3.0 GPS. Average values of E_{vd} on forest roads ranged from 29.5 MN/m^2 to 50.4 MN/m^2.

Key words: *forest roads, road construction, bearing capacity, dynamic module of deformation, Evd,*

Introduction

Forest roads are the most important infrastructural facilities in the realization of works in forestry. At the same time, the construction and maintenance of forest roads are the most expensive activities (*Ranković & Butulija, 1998*). Forest roads are planned, designed and constructed infrastructural facilities, whose spatial and construction solution depends on the shape of terrain, the purpose of forest, habitat and stand conditions of forest ecosystems. (*Stefanović, et al. 2016*). In Serbia, forest roads are most commonly built as single-track roads with a road width of at least 3,0 m. The construction of forest road pavement represents the most demanding works, which has to take place in appropriate weather conditions and demands the application of appropriate mechanization and construction technology. The forest road pavement is a specially built part of forest road on which vehicles move. It's task is to receive the load from traffic that takes place on it and transfer that load to the lower road contruction elements. The pavement has to be resistant to the atmosferic influences (rain, snow, frost, sun, etc.), but also to endure the anticipated load and traffic intensity.
In order to fulfill all the required durability conditions, the pavement has to be built of materials of appropriate quality, on well-prepared surface (earth surface), respecting the

principles and technology of forest road construction. The use of crumbled stone aggregate, which has a certificate of quality given by accredited laboratories, will undoubtedly provide pavement of good load-bearing and durability. However, the use of such materials significantly increases the total cost of construction of forest roads. High purchase price of crumbled stone aggregate is not the only cause, but also (mostly) large transport distance between the quarry and the forest road. For that reason, stone aggregates that can be found near the forest road is most commonly used for the contruction of forest roads. Preparation of such stone aggregate is carried out by excavators with depthe bucket, hydraulic hammer or blasting and crushing into appropriate fractions. The practice of testing the degree of compaction of different layers of pavement is present in road construction for a long time, but it has not found wider application in the forest road contruction, except in individual cases. Today, there are varios devices for the examination of the degree of compaction of materials. The most of those are static and require counterweight, as well as well-trained workers to handle them. In addition, in recent years, simpler instruments have been developed – dynamic plates with falling weight, for determining the degree of compaction of different types of materials via dynamic deformation modulus (E_{vd}).

Considering the modest experience in measuring the compaction of poured materials on forest roads, the aim of this papre is to point out the importance of such tests. The paper presents the examination results on four freshly built forest roads, whose pavement structure is built of locally available stone aggregate.

Research area

The paper presents the results of testing the dynamic modulus of deformation on four freshly built forest roads in different parts of Serbia. The examination was performed on the following forest roads:

1. Forest road „Valja rea" in FMU „Brodica", Forest holding „Severni Kučaj" Kučevo;
2. Forest road „Vrba – Potok Almari", in FMU „Crna reka", Teaching base „Majdanpečka domena", Universiti of Belgrade – Faculty of Forestry;
3. Forest road „Beočić – Bela Grača – Zmajevica", in FMU „Juhor 1", Forest holding „Južni Kučaj" Despotovac and
4. Forest road „Lovački dom – Milojkovača", in FMU „Bukulja", Forest holding „Kragujevac" Kragujevac.



Materials and methods

The examination of compaction of pavement of forest roads was performed with ZORN device, type ZFG 3.0 GPS. This simple device with falling weight presents a fast method for determination of dynamic modulus of deformation E_{vd} [MN/m^2]. Dynamic modulus of deformation E_{vd} is a parameter of ground deformation under vertical load impact. It allows drawing conslusions about bearing capacity and ground compaction. A version of the device with a weight of 10 kg is used to measure compaction of earth materials in embankment and

subgrade, as well as loose stone materials up to 70 MN/m^2. For measuring the compaction of highly compated materials, a version of device with weight of 15 kg is reccomended. A 50% heavier falling weight provides accurate measurement up to 105 MN/m^2.

The examinations of dynamic modulus of deformation were conducted in 2019 on four forest roads that were built in the same year. Forest roads were designed in accordance with the principles of forest road designing, and their construction was monitored by appropriate professional supervision.

For the contruction of pavement on forest road "Valja rea" a natural stone aggregate from the route of the forest road was used. Laboratory tests of the mineralogical-petrographic composition have determined this stone as surface decomposed shales. Measurements of the dynamic modulus of deformation were conducted at five freely selected points along the road. The road was mostly built on a ground that belongs to the category IV and a smaller part to category V. At the time of measurement, the weather was cloudy, without rain, but the pavement was wet, since it was raining the day before.



For the contruction of pavement on forest road "Vrba – Potok Almari" a natural stone aggregate from the route of the forest road was used, also determined as surface decomposed shales. The road was built on a ground that belongs to the category IV. Measurements of the dynamic modulus of deformation were conducted at five freely selected points. At the time of measurement, the weather was cloudy, without rain, and the pavement was mainly dry.

For the contruction of pavement on forest road "Beočić – Bela Grača - Zmajevica" a natural stone aggregate from the borrowing point at the route of the forest road was used. According to mineralogical-petrographic composition it was determined as surface decomposed peridotite. The forest road route was mostly along the ground that belongs to the category III and IV. At the time of measurement, the weather was cloudy, without rain, and the pavement was mainly dry. Measurements of the dynamic modulus of deformation were carried out at five points.

A natural limeston stone aggregate was used for the contruction of pavement on the forest road "Lovačka kuća – Milojkovača". Borrowing point was located near the forest road. Before the exploatation of natural stone the blasting had been carried out. The stone aggregate was not crumbled, but used in natural state. The road was mostly built on a ground that belongs to the category III. At the time of measurement, the weather was fine, without rain, and the pavement was dry. Measurements of the dynamic modulus of deformation were carried out at ten points.

Results

Tests of the dynamic modulus of deformation on the pavement of forest roads show a great heterogeneity of the obtained results.

The values of dynamic deformation modulus on forest road "Valja rea" ranged from 22.3 (at the forest road turntable) to 37.9 MN/m^2, and average 29.5 MN/m^2. The value of s/v in all measurements had high values, which indicates that further compaction can provide a higher value of dynamic modulus of deformation E_{vd}.

Table 1: E_{vd} and degree of compaction on forest road "Valja rea"

No.	Coordinates (Google Earth)		E_{vd} [MN/m^2]	s/v
	X	Y		
1	44°33,907'	21°51,288'	23,5	4,852
2	44°33,908'	21°51,288'	22,3	6,080
3	44°33,885'	21°51,299'	27,7	5,065
4	44°33,277'	21°51,135'	35,9	4,354
5	44°32,674'	21°50,919'	37,9	4,156
		AVERAGE	**29,5**	**4,901**

The examination of dynamic modulus of deformation on forest road "Vrba – Potok Almari" was carried out on five freely selected points and the measured values ranged from 44.6 MN/m^2 at the end of forest road (turntable) to 58.158,1 MN/m^2. The values of s/v within all measurements were bellow 3.5, which indicates that further compaction would not provide a higher value of dynamic modulus of deformation.

Table 2: E_{vd} and degree of compaction on forest road "Vrba – Potok Almari"

No.	Coordinates (Google Earth)		E_{vd} [MN/m^2]	s/v
	X	Y		
1	44°20,897'	21°53,795'	48,4	3,077
2	44°21,059'	21°53,701'	58,1	2,934
3	44°21,181'	21°53,590'	47,6	2,799
4	44°21,343'	21°53,777'	53,1	2,762
5	44°21,288'	21°54,330'	44,6	2,812
		AVERAGE	**50,4**	**2,877**

The values of dynamic deformation modulus on forest road "Beočić – Bela Grača - Zmajevica" ranged from 18,20 to 59,80 MN/m^2. The value of s/v in some measurements was significantly higher than 3.5, which indicates that further compaction can provide a higher value of dynamic modulus of deformation (higher level of compaction).

Table 3: E_{vd} and degree of compaction on forest road "Bečić – Bela Grača - Zmajevica"

No.	Coordinates (Google Earth)		E_{vd} [MN/m^2]	s/v
	X	Y		
1	43°49,001'	21°12,495'	24,30	3,563
2	43°49,004'	21°12,480'	39,90	3,214
3	43°49,148'	21°12,428'	59,80	2,659
4	43°49,331'	21°12,513'	18,20	6,857
5	43°49,321'	21°12,501'	29,70	3,584
		AVERAGE	**34,4**	**3,975**

The values of dynamic modulus of deformation on forest road "Lovački dom – Milojkovača" ranged from 41.0 to 61,8 MN/m^2, and average of 49,7 MN/m^2. The values of s/v within all measurements were bellow 3.5, which indicates that further compaction would not provide a higher value of dynamic modulus of deformation E_{vd}.

Table 4: E_{vd} and degree of compaction on forest road „Lovački dom - Milojkovača"

No.	Coordinates (Google Earth)		E_{vd} [MN/m^2]	s/v
	X	Y		
1	44°17,414'	20°31,425'	61,8	2,463
2	44°17,475'	20°31,344'	51,0	3,369
3	44°17,468'	20°31,214'	41,0	2,963
4	44°17,458'	20°31,009'	46,4	3,009
5	44°17,415'	20°30,882'	48,3	2,67
		AVERAGE	**49,7**	**2,89**

Discussion and conclusion

Forest roads are characterized by low intensity of traffic over time, but it significantly increases during the period of tree felling and during those periods forest roads put up with high loads produced by mechanized vehicles. Dimensioning of the forest roads pavement is carried out an accordance with the category of the ground, without previous geotechnical tests. On higher ground categories (V, VI, VII) forest roads are built with a pavement thickness of 0.15 – 0.20 m, while on lower ground categories (I, II, III) it is often greater than 0.50 m. The pavement of forest roads is made of different types of stone aggregate, which most often implies natural or separated gravel and natural or crumbled stone. The pavement construction is one of the most expensive phases within forest road construction, especially in lowland conditions, where pavement construction participates with up to 80% in total construction price. Analyzing 71 major projects involving the construction of 178 km of forest roads, Dražić et al. (2020) found out that pavement construction participates with 40% in total forest road construction price in hilly-mountainous area of the Republic of Srpska. Considering the high construction cost of forest road pavement, but also it's importance for the load-bearing capacity and durability of forest road, special attention should be paid to the forest road pavement. The analysis of the obtained results showed that there was a big difference amoung the obtained values of the dynamic modulus od deformation (E_{vd}), which is particularly noticeable at forest roads "Valja Rea" and "Vrba – Potok Almari" that were built in the approximately same conditions and are made of the same stone aggregate (Table 5). When observing the values of s/v for all four forest roads, it is noticed that performed compaction of pavement (and probably the subgrade) on forest roads "Valja rea" and "Beočić – Bela Grača – Zmajevica" was not enough and and that it is possible to reach higher values of dynamic modulus of deformation by additional compaction. A surprising results were obtained on forest road "Lovačka kuća – Milojkovača", with a pavement width of 0.5 m, made of natural limestone stone aggregate, which is also used for the construction of bearing layers of modern roads. The average values of the dynamic modulus of deformation on this road were 49.7 MN/m^2 and the most probable cause was the insufficiently compacted road subgrade.

Table 5: Comparison of influencing factors and E_{vd} test results on forest roads

No.	Forest road	Ground category	Stone aggregate	E_{vd} [MN/m^2]	s/v
1	Valja rea	IV-V	Shales (surface decomposed)	29,5	4,901
2	Vrba – Potok Almari	IV	Shales (surface decomposed)	50,4	2,877
3	Beočić – Bela Grača - Zmajevica	III-IV	Peridotite (surface decomposed)	34,4	3,975
4	Lovačka kuća - Milojkovača	III	Limestone	49,7	2,890

It is assumed that the measured values of the dynamic modulus of deformation were, to some extent, affected by the humidity of the pavement and the subgrade, but it was not examined in this case. Grajewski (2016) states that the bearing capacity of the pavement varies depending on the amount of moisture in the bearing layers.
The results of these initial researches indicate that researches should be continued on a much larger scale and on a larger sample. Also, the researches should include a larger number of types of stone aggregates and other factors that can affect the compaction of pavement should be determined. Such researches could have a great influence on increasing the quality of performed works within forest road contruction, thus the bearing capacity and durability of the forest roads.

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STRUCTURAL AND PRODUCTION CHARACTERISTICS OF THE MOST IMPORTANT FOREST TYPES IN FOREST ENTERPRISE "VISOCNIK" (BOSNIA AND HERZEGOVINA)

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Abstract

The paper presents comparative analyzes of the tree number, volume and height of edificators (fir, beech and spruce) in three different production forest types (managemet classes). The aim is to study the production and structural characteristics of the various types of forests in investigated area of Visocnik. Primary data of taxation was collected (DBH above 10 cm and three heights as well) for all trees with DBH above 10 cm as their heights as well. After that the stand volume was calculated. According to this results of the research, the height curve was to the most homogeneous. The lowest heights were recorded by European beech in the investigated production forests types (management classes), while similar height curves were determined for spruce and fir. The height curve was deviates at most in the management class 1210 (forest type of European beech and fir on a series of limestone predominantly deep soils). The height was from 11m to 34m. In management class 1210 was recorded the highest number of fir tree as well as volume. Tree number and volume are more uniform for management class 1208 and 1209. In this management class the differences are determined for European beech. The number of Eueopaean beech trees is 16 and the volume is 23,08 m³. The natural rejuvenation is the most perfect form of the regeneration of the stands because the stand regenerated in that way contains the continuity of stability and productivity inherited by the former mother stand.

Key words: *Fir, Spruce, European beech, management classes*.

Introduction

According to the data of Forest cadastre and forest land (2009) the total area of forests and forest land of the Entity of Republic of Srpska (RS) in Bosnia and Herzegovina amounts to 1.282.412 ha i.e. 51,7% of total area of the RS. The total area of high forests of Forestry Industry Area ''Hanpjesačko'' amounts to 17.068,78 ha which represents 93,65% of the area of the forestry industry area. The structure of wood stock, as the most important types of trees, consists of: fir with mixture ratio of 48,05%, spruce with mixture ratio of 32,83% and European beech with mixture ratio of 15,37%. The category of the high forests with natural regeneration takes up 456.674 ha or about 46,95% of the total area of the forestry fund of the RS. Mixed forests of beech-fir and fir-spruce-beech mostly provide stands of selective or multi-aged structural shape, with medium to high productivity. The most productive forests in the Republic of Srpska are beech, fir and spruce forests (Govedar, 2016). The group–selection system management is the most used one in these forests (Govedar, 2016), the importance of these forests is reflected, as it is known, in the structural and speciation diversity. Knowing the structural and production characteristics provides the estimation of the stability of the communities and for the mentioned reason the analysis of the most common management classes has been conducted in this paper. The reason for the direction of these

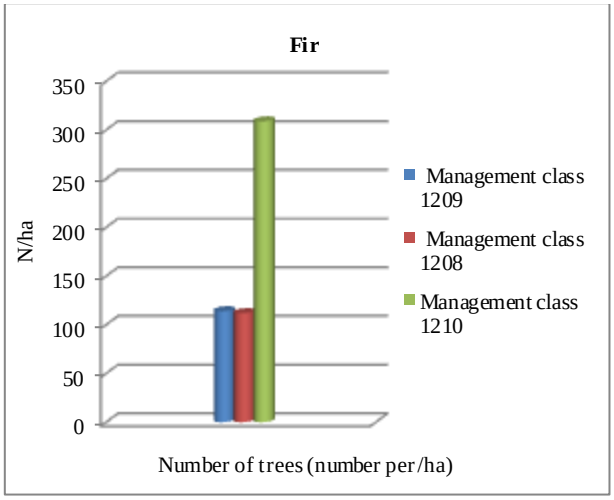
researches was due to the appearance of the potential cases of drying and reduction of the share of conifers in these communities (Parobekova, 2018).
The aim of this paper is to study the production and structural characteristics of the various types of forests at the area owned by FE ''Visočnik'' Han Pijesak.

Material and Methods

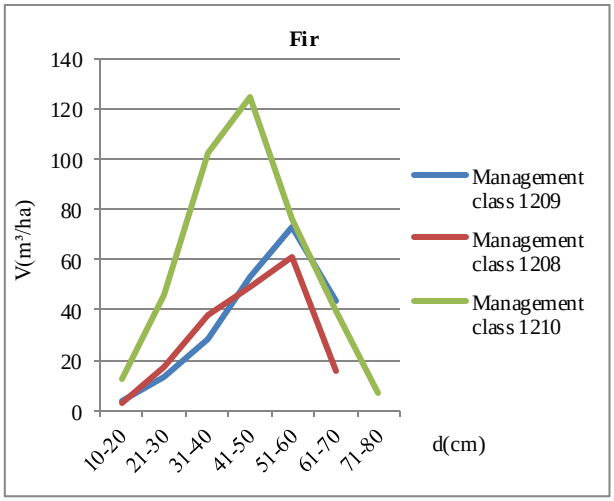
Determining of the structural and production characteristics of the mixed forests of Eureopean beech, fir and spruce was conducted at three sample areas in the management classes 1208, 1209 and 1210. The collection of the primary taxation data at the set sample areas was conducted by having all the trees with DBH above 10 cm surveyed and their heights as well. After that the stand volume was calculated.
The management class (1209) are European beech, fir and spruce forests at the series of chalky mostly shallow soils and in the combination of mostly shallow soils with dystric cambisol (acid brown soil) on siliceous carbonate rocks. The management class (1208) are European beech and fir forests with spruce on deep acid brown soils, on acid siliceous rocks and on the soil in the combination of mostly deep acid brown soils with shallow chalky soils on the siliceous carbonate rocks. The management class (1210) are European beech and fir forests with spruce at the series of chalky mostly deep soils. The phytocoenological record was made pursuant to the *Braun–Blanquet* method.

Results and Discussion

The first of all we will show comparative analysis of structural and production characteristics. The graph 1 shows the visible domination of the number of fir trees at the management class 1210 contrary to the other two sample fields. Fir stocks at the management class 1210 are large due to the fact that the regular cutting has not been done in the last management period due to the possibility of having excess of wood stock in this management class. On the graph 2 we can see that at the management class 1209 fir has the largest volume whose culmination is about 45 cm in DBH. Concerning the other two fields, the diameter structure of the stand is stronger with greater share of thick trees and culmination of the volume which is almost identical, at about 65 cm in DBH.

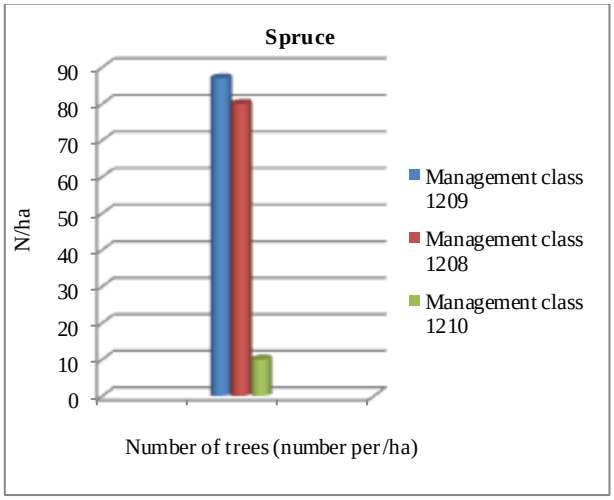


Graph 1. Display of the number of Fir for different management classes

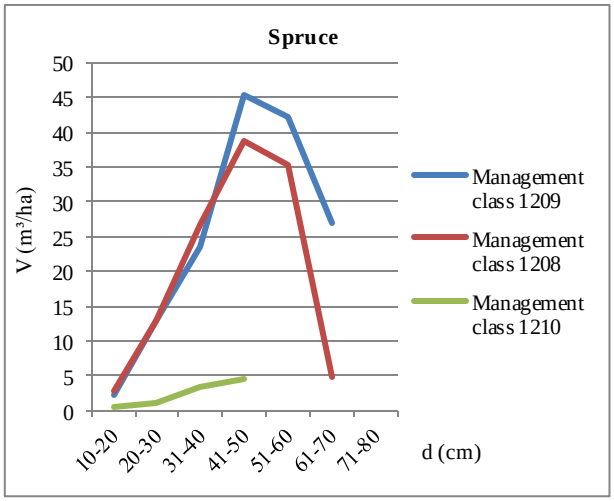


Graph 2. The distribution of the volumes of the fir trees for different management classes in relation to DBH

The graph 3 shows that the number of trees concerning the management class 1209 and management class 1208 is approximately equal while in the management class 1210 the number is abnormally low. The reason for that is the sanitary cutting of spruce trees in small and large groups by which the distribution of space has been disturbed in some parts of the sections. On the graph 4 we can see that the spruce as a type of tree is considerably more found in management classes 1209 and 1208 with almost identical culmination between 40 and 60 cm in DBH.

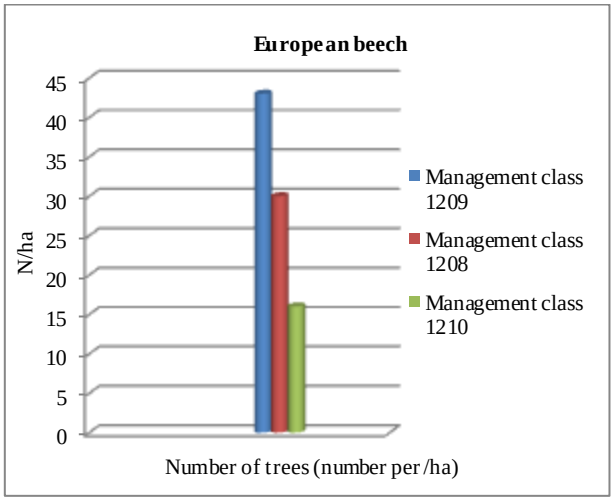


Graph 3. Display of the number of spruce for different management classes

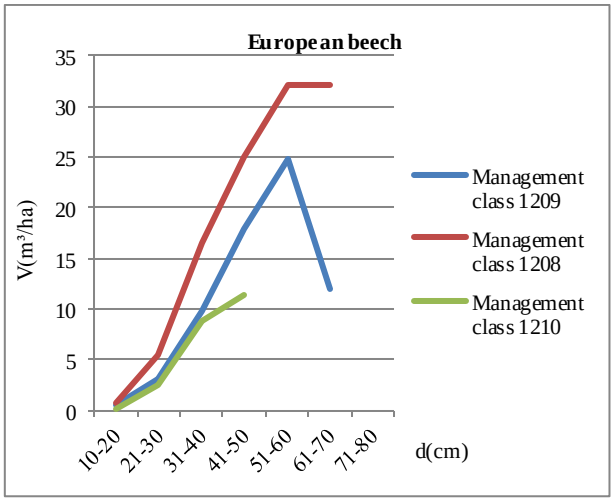


Graph 4. The distribution of the volume of the the spruce trees for different management classes in in relation to DBH.

On the graph 5 we can see that the number of European beech trees is naturally found at the management class 1209 while it does not display the real state in the other management classes by comparison to the one on the field and by management elaborate. The graph 6 shows that concerning the beech as a type of tree at the management class 1210 a small number of trees is found, mostly the ones of small volume. At the other two management classes, the European beech has a quality volume with the culmination of about 55 cm in DBH. At the sample management class 1208 the volume of the European beech trees is equally distributed in the range of 55 cm to 65 cm in DBH.

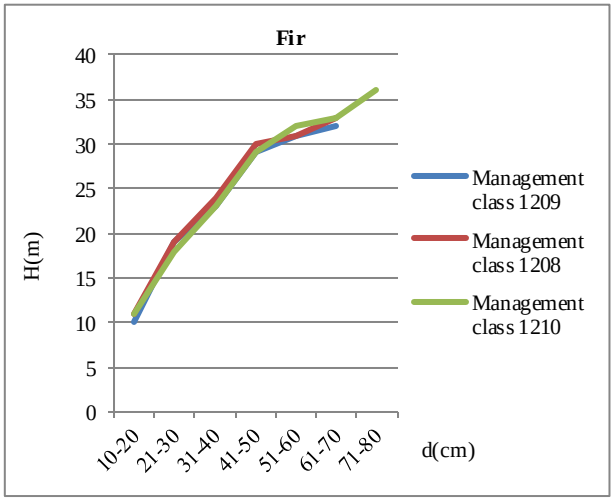


Graph 5. Display of the number of European beech for different management classes

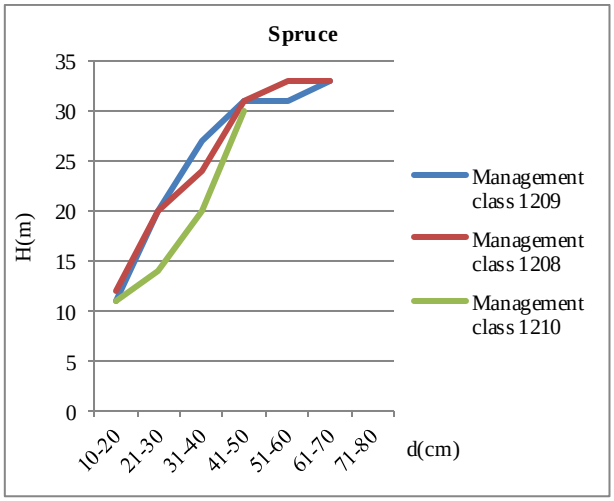


Graph 6. The distribution of the volume of European beech trees for different management relation to DBH

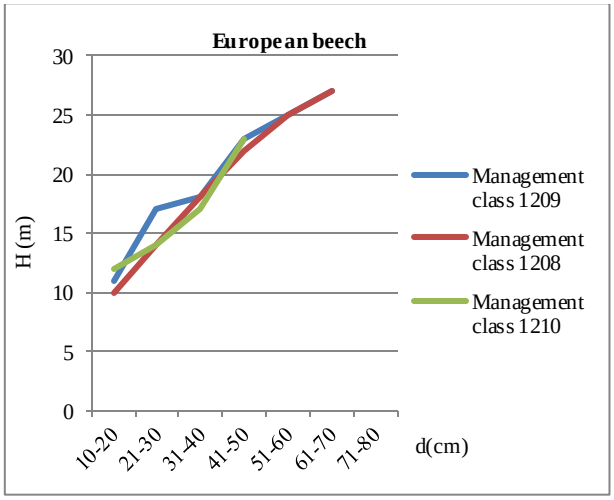
On the graph 7, 8 and 9 we can see the height curve three different species of trees in different management classes. On the graph 7 we can see that the heights of the fir trees mostly coincide at all sample fields with no expressed oscillations. The 8th graph shows that the height curve of the spruce trees, along with minor deviations, overlap at the management classes 1209 and 1208, while at the management class 1210 the heights of the spruce trees are considerably lower with the increase of DBH. At the 9th graph we can see that the height curve of the beech trees overlap mostly in all management classes.



Graph 7. The height curve of the fir for tree different management classes per diameter degrees



Graph 8. The height curve of the spruce for different management classes per diameter degrees



Graph 9. The height curve of the beech for different management classes per diameter degrees

The height curve of the European beech trees overlaps mostly at all the sample fields. It is well-known fact that the fir, European beech and spruce react differently to the shade which requires different processes in conducting selective cutting with the purpose of rejuvenation and ingrowth of these tree species. The European beech can bear the shade for a shorter period than the fir tree can and that surely should be taken into the consideration in choosing selective cutting (single- tree, group selection).

The height structure provides better insight into the vertical development of the stand, i.e. provides more light in the structural characteristics of the selection forest (Miletić, 1954). In the typical selection forests, the distribution of the number of trees per height degrees should have the same primary shape as the distribution of the number of trees per diameter degrees, i.e. to have the largest number of trees in the lowest height degrees which should gradually decrease towards higher heights (Leibundgutm 1945). The selection forests with vertical development are rarely found in the nature, but there are mostly various transitional forms of vertical development between even-aged and selection stands. The reason for distance from the height structure, specific for selection forests in the stands of these sites, is that the vertical composition and staggered development are lost by large wood stocks. When the selection

forests with considerable volume are concerned, the construction per layers is more homogenous and the vertical space is filled better (Miletić, 1950).
Movement of the height curves is directly connected to the changes of height structure of these stands during the time. If the structure of the selection forest deviates from the balanced state only in certain diameter degrees, the rise of heights in them occurs (Banković, 1971). The movement of the height curves is considered to be a normal phenomenon in the selection forests which are not in the balanced state. It means that each structural disorder and distance from the balanced state contributes to the movement of the height curves and vice versa, approaching the typical selection structure leads to the decrease of this phenomenon in the selection forests, which was confirmed by this research.
The volume is the derived structural element in which the influence of most other elements and their distributions has been expressed. In analyzing the volume of the selection forest, its size per hectare is very significant as well as its distribution per diameter degrees (classes)– the volume structure. The size of the volume per hectare at selection stands moves in very wide extents and it depends on the type of the trees, mixture ratio, quality of the habitat, diagram of cutting maturity and as well on the number of trees and their distribution per diameter degrees. The movement of wooden volume on the log, as one of the more significant elements of the constitution of the selection stand, is a very good indicator of the success in management, development and state of the stands (Čavlović, 1997). In respect of the importance of the volume, from the productive and exploitative aspect and the aspect of revising the effects of the management treatment in the past, the special attention was dedicated to it during these researches. The volume structure, according to the types of the trees is irregular, bell-shaped in the base, with right asymmetry which increases during the time, with the increment of the width of the variation as the consequence of the movement of the inventory towards stronger diameter degrees.
One of the most difficult tasks of the selection management is to achieve and preserve the typical selection structure permanently. However, the selection system should be considered as something that is still in development and all in the spirit of adaptive (adjustable) management (O'Hara *et al*., 2007). It has also been recognized as one of the most favorable system of management (single-tree and group selection) when the reactions of the forest to the climate changes are concerned (Brang, 2014).
The aim of this system is to promote the structure and the composition of the types of trees which use the conditions of the habitat (the environment) best. It is not real to except the structure and the composition of the types of trees of these forests to remain unchanged during the time. The flexibility of the selection system gives the opportunity for correction and guidance of the changes to the wanted direction. Having concerned that the selection system was made by a human and it can be in function as direction.
Having concerned that the selection system was made by a human and it can be in function as long as the structure of the stands is improved by periodical interventions (Schütz, 1999), as even the balanced selection forest with optimal structure and vertical constitution without having selective cutting, left to self-regulative processes (spontaneous development), gradually impoverish in trees of low and medium floors and becomes single-layer structure with horizontal constitution.

Conclusion

The comparative analyses of the number of the trees, volume and height of the edificators (fir, European beech and spruce) in three different production types of the forest (management class) have been carried out in this paper. According to the results of the research the height curve has shown to be the most homogenous. In the studied production types of the forests

(management classes) the lowest heights were recorded in beech trees whereas the height curves of fir and spruce are approximately the same. The height curve has the highest deviation in the management class 1210 i.e. in the beech and fir forest at the series of chalky mostly deep soils. In the management class 1210, the largest number of fir was recorded as well as the volumes of the same type of the tree. The number of trees and volume in productive types of forests (management classes) 1208 and 1209 is more homogenous when fir and spruce are concerned whereas the number of trees and volume of European beech was the lowest. The natural rejuvenation is the most perfect form of the regeneration of the stands because the stand regenerated in that way contains the continuity of stability and productivity inherited by the former mother stand. European beech and fir have heavy seed so their rejuvenation is possible only near the tree. The spruce has small seed which can be easily passed and provides rejuvenation at longer distances from the tree. The enlightened places between the European beech and fir trees in the forest and as well the more humid land lots suit the spruce. The sprout of beech and fir is sensitive to low and the one of the sprout to high temperatures. The sprout of the spruce is resistant to the frost.

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GENETIC DIVERSITY OF NORWAY SRUCE (*Picea abies* (L.) Karst.) IN NATIONAL PARK TARA (SERBIA)

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Abstract

The results of genetic variability analysis of natural Norway spruce (*Picea abies* (L.) Karst.) population located in the National park Tara in western Serbia obtained using SSR (Simple Sequence Repeat) markers have been shown in this paper. The use of microsatelite markers become tool of choice for population studies in the genetic characterization of different tree species. The genomic DNA has been isolated from needles tissue of 30 mature individuals.
Out of 11 SSR primer sets markers eight (72.7%) were successfully amplified clear reproducible and polymorphic alleles among analyzed individuals of Norway spruce population. Total number of alleles revealed for all analyzed individuals were 57, with the average number of alleles 7.1 per locus. The number of alleles obtained with different SSR primers varied from eleven (EAC1F04) to five (WS0092.M15). Genetic similarities were calculated in NTSYSpc2.1 program package by Dice's coefficient. Relatively high genetic variation was observed among individual genotypes of population, as genetic distances were in range from 0.15 to 0.55. Illustration of genetic structure of natural Norway spruce population was obtained using the selected SSR markers. The necessary measures for the conservation of genetic resources in the studied population and adoption of management and renewal plans can be recommended based on the obtained results.

Key words: *Picea abies* (L.) Karst., SSR, population, variability.

Introduction

The high level of diversity in terms of genetic resources, abundance of species and habitat diversity classifies the forests among the most complex ecosystems on Earth (Geburek and Konrad 2008). The biodiversity is endangered due to the constant pressure on forests in the several last centuries and disappearance of natural forests (Carabeo et al. 2016).The long-term survival of species is closely related to their genetic diversity (Gapare 2014). Under the influence of changed environmental conditions, biotic agents causing diseases and damages the survival and evolution of species depend on the level of genetic diversity (Reed and Frankham 2003). The research dealing with genetic diversity and identifying populations characterised by high genetic variability can help in reduction of risk of biodiversity loss (Souto et al. 2015). Considering the climate change, genetic diversity is one of the most important factors that can contribute to adaptability and survival of species (Thompson et al. 2009).
Norway spruce (*Picea abies* (L.) Karst.) is one of the most significant ecological and commercial tree species in Europe with very pronounced variability (Koski et al. 19997). Considering the above, the studies of genetic variability in the past were very intensive (Giannini et al. 1991; Geburek 1999; Muller-Starck 1995; Goncharenko et al. 1995; Konnert 2009; Longauer et al. 2001; Božič et al. 2003; Ballian et al. 2007a, 2007b; Korshikov and

Privalikhin 2007; Kravchenko et al. 2008; Teodosiu 2011; Sabor et al. 2013). The studies by using molecular markers have shown great genetic variability of spruce in Europe and the phenomenon of great differentiation among populations (Muller-Starck et al. 1992). Under certain ecological conditions, spruce is in the constant process of genetic differentiation and adaptation (Langercrantza and Rymana 1990). In some studies it was concluded that this is the species that by means of genetic structure of population reacts very well on changes of ecological conditions of habitat, primarily the changes in the soil (Božič 2002), as well as pollution of air and soil (Bergman and Hoisus 1996; Longauer et al. 2001).
The objective of research in this paper is to examine genetic diversity of spruce in the population on Tara Mountain using modern molecular genetic methods. The obtained results may serve for making recommendations in implementing the measures necessary for conservation of genetic resources and adoption of management and natural forest regeneration plans.

Materials and methods

The research was conducted in the natural population of spruce on Tara Mountain (Table 1). During August 2018, twigs with needles were collected from 30 representative trees over 70 years old. The distance between the trees was between 50 and 80 meters in order to exclude the possibility of affinity.

Table 1. Geographical characteristics of the studied population

Population	Latitude	Longitude	Elevation (m)
NP Tara	43° 54' 58"	19° 26' 41"	1000-1160

Thirty mature individuals of Norway spruce (*Picea abies* (L.) Karst.) were characterized using 11 Metabion SSR markers (Table 2). Needle tissue was ground in a mixer mill Qiagen TissueLysser for 2 x 3 min at 29 Hz and genomic DNA has been isolated from following modified CTAB method according to Doyle and Dolyle (1987). PCR amplifications for each SSR marker were conducted in a total volume of 20μL using 2.5μL reaction buffer (10x), 2μL nucleotide mix (10mM), 0.2μL Taq polymerase, 0.25μL of of each primer equences (forward and reverse - 0.5μM) and sterile, distilled water up to 19μL and then 1μL of genomic DNA was added. All amplifications reactiones were performed in Biometra TProfessional Standard Thermocycler using following programme: initial denaturation at 95ºC for 10 minutes, followed by seven cycles of touchdown (30 s at 94ºC each, 30 s annealing of the primers at temperatures from 57ºC to 60ºC with 1°C decrement per cycle, 30 s at 72°C), 28 cycles of amplification (45 s at 95ºC, 45s at anneling temperature (Table 1), 45s at 72 ºC) and final extension phase (10 min at 72 ºC).

Table 2. List of used primer sequences, repeat motifs and anneling temperature

Locus	Forward primer	Reverse primer	Repeat motifs	Ta (°C)
WS00716.F13	tcaagtaatggacaaacgataca	tttccaatagaatggtggattt	$(GA)_{10}$	50
WS0092.M15	gatgttgcaggcattcagag	gcaccagcatcgattgacta	$(TCC)_6$	53
WS0022.B15	tttgtaggtgctgcagagatg	tggctttttattccagcaaga	$(AG)_{12}$	50
WS0073.H08	tgctctcttattcgggcttc	aagaacaaggcttcccaatg	$(AT)_{14}$	51
WS00111.K13	gactgaagatgccgatatgc	ggccatatcatctcaaaataaagaa	$(AT)_9$	53
WS0023.B03	agcagctggggtcaaagtt	aaagaaagcatgcatatgactcag	$(AT)_{10}$	52
WS0046.M11	cactagggcattgggaagaa	atgagaggctggggtatgaa	$(AAG)_6$	53
EAC1F04	tgtaagtctgcttgaaggtgg	cagatgggggttgggtat	$(AC)_{34}$	51
Pa 44	aaggcagccaaagtgaagaa	cttggcattccctagtgagc	$(GGA)_n$	51
Pa 51	cagatgtgggcacttgtttg	tggtcatggtggtgttcat	(CCA)n	51
Pa 47	atcaattgccctaccagcac	tgctcaatttcctgcatctg	(CAG)n	51

Ta- anneling temperature

The gel images of SSR data results were scored using GelAnalyser (Version 2010a). The binary data matrix was formed based on presence (1) or absence (0) of specific alleles for each locus. For each SSR locus polymorphism information content (PIC) was caluclated for the purpose of assessing the informativnes of each locus. PIC = $1 - \sum(pi2)$ where pi is number of alleles / number of genotypes. Genetic similarities (GS) between analyzed genotypes were calculated in NTSYSpc2.1 program package by Dice's coefficient.

Results and Discussion

Microsatellite markers might be useful tool in population genetics studies and breeding of different tree species. All analysed samples in this paper were genotyped initially by 11 SSR loci described in several studies (Rungis et al., 2004; Scotti et al., 2002; Fluch et al., 2011). Primers developed by Rungis et al. (2004) are EST-SSR markers which are frequently used since their transferabillity across spruce. Scotti et al. (2002) and Fluch et al. (2011) published genomic SSR sequences used in numerous studies of genetic variation of Norway spruce. Di and trinucleotide microsatellite markers developed in mentioned works become tool of choice for population studies of the spruce genome.

Out of the 11 SSRs tree were not included in data analyses since they were monomorphic. The amplification of remaining number of SSR markers detected high level of poymorphism, as the number of detected alleles per locus varied between five (WS0092.M15) and eleven (EAC1F04), with mean value of 7.1 and tolal of 57 allelic variation. Lower mean number of alleles (5.6) was observed with 23 SSR primers on 96 individuals of Norway spruce (Fluch et al., 2011). On the other hand, 8.1 polymorphic alleles per locus were found in work of Galovic et al. (2015) with 16 primer sets on five genotypes.

Table 3. List of used primers, allele size range, repeat motifs, PIC, number of alleles

Locus	Allele size range (bp)	Repeat motifs	PIC	Number of alleles
WS0092.M15	205-220	$(TCC)_6$	0.76	5
WS0022.B15	170-215	$(AG)_{12}$	0.48	8
WS0073.H08	205-250	$(AT)_{14}$	0.38	7
WS00111.K13	185-225	$(AT)_9$	0.51	7
WS0023.B03	170-205	$(AT)_{10}$	0.45	7
EAC1F04	181-371	$(AC)_{34}$	0.54	11
Pa 51	120-140	(CCA)n	0.61	6
Pa 47	90-120	(CAG)n	0.51	6
Mean			0.53	7.1

Among eight SSR primers polymorphic information content (PIC) values ranged from 0.38 (WS0073.H08) to 0.76 (WS0092.M15), with an average of 0.53 (Table 3). Variation of the PIC values depends on several factors as the type of SSR markers (dinucleotid, trinucleotid) and analyzed genotypes (number or samples, genetic nature). In work of Binowa et al. (2020) mean PIC value was 0.67 for 16 SSR markers.

As expected relatively high genetic variation was observed among individual genotypes of population, as genetic similarities values were in range from 0.15 to 0.55. The average value of the genetic similarity between analysed individuals was 0.29. Also, Dice's genetic distance matrix showed high values between individuals of Noraway spruce, ranging between 0.79 to 1.00 (Galović et al., 2015). In contras low values of genetic distance (0.00- 0.032) between Serbian populations are found in work of Stojnić et al. (2019). This is consistent with earlier findings that intapopulation variability is higher than between different populations.

The high intrapopulation variability was determined with populations in Bosnia and Herzegovina (Ballian et al. 2007a). Furthermore, great intrapopulation variability was determined on the narrow area on Igman Mountain (Ballian et al. 2007b). A significant genetic variability was also determined with some populations of Romanian Carpathians

(Radu et al. 2014) and Ukrainian Carpathians (Korshikov and Privalikhin 2007). It was found that the level of intrapopulation genetic variability was higher and the level of interpopulation genetic variability decreased (Lewandowki and Burczyk 2002; Korshikov and Privalikhin 2007; Goncharenko et al. 1995; Ballian et al. 2007a; Kravchenko et al. 2008; Radu et al. 2014). In many studies vertical differentiation was determined in spruce. (Krajmerova and Longauer 2000). Lower values of genetic diversity were determined in the populations of Western Europe (Lagercrantz and Ryman 1990; Mánec 1999; Krajmerova and Longauer 2000) compared to the populations from the Balkan Peninsula (Ballian et al. 2007b; Milovanović and Šijačić-Nikolić 2010). The research has shown that Balkan populations have not lost much of their genetic potential for adaptation, so it can be assumed that they are more resistant to decay and for survival because they possess sufficient genetic variability (Ballian et al. 2007a).

Conclusion

The results obtained in this research have shown high level of genetic variability on the level of researched population. The satisfactory genetic diversity recommends this population for the dynamic conservation of genetic resources of spruce in Serbia. For the purpose of additional security of gene pool conservation it is recommended to establish ex situ conservation objects. Considering the pronounced genetic variability, it can be assumed that the studied population has the resistance to decay and adaptive potential regarding changes caused by climatic conditions.

In management of natural populations of spruce it should be insisted on measures that would ensure natural regeneration along with the constant monitoring of genetic structure and implementation of the timely measures to maintain natural genetic diversity.

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NATURAL AND CULTURAL LANDSCAPE MANAGEMENT IN NAHR EL KALB RIPARIAN FOREST IN LEBANON

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Abstract

Riparian forests in Lebanon are very rich in biodiversity due to the climate and topography of the country. Nahr el Kalb historical river presents riparian forests rich in natural and cultural resources and is in danger of disturbance due to uncontrolled constructions. The objective of this study is the conservation of Nahr el Kalb's riparian forest through landscape management aiming for ecotourism. The method used is site visits to identify natural and cultural resources of riparian forests: understanding locals' perceptions using the Knowledge Attitudes Practices (KAP) method, followed by a Strengths Weaknesses Opportunities Threats (SWOT) analysis and specifying potential ecotourism elements. Finally, a landscape master plan of the riparian forest with sketches explaining ecotourism activities. Results show that the steep slope of natural diversified layered vegetation, covered water channels along the river, orange trees orchards, and old bridges and buildings are potential elements for ecotourism. Locals are aware of the importance of these resources and are willing to volunteer in the management process. The conservation landscape plan presents three sustainability pillars. Ecologically, the existing covered water channel is used as a walking path after weed clearing with informative signs. Socially and economically, transformation is needed of the old buildings into guesthouses and forest-related museums with the preparation of an annual festival during orange season where eco-tourists can participate in orange picking and buy local products. In order to conserve natural and cultural riparian forest resources in Lebanon, ecotourism should be used in the landscape management plan of these forests.

Keywords: *riparian forest, ecotourism, landscape management, natural and cultural resources, Lebanon.*

Introduction

Riparian forests consist of important areas rich in natural and cultural resources. Depending on the topography and climate, they consist usually of undisturbed landscapes due to their inaccessibility for humans. When in danger inflicted by the uncontrolled constructions that might cause damages to the riparian forest, ecotourism plays a sustainable role in its conservation (Osman et al., 2012), since one of its main principles is to minimize the impact on natural resources (Bricker, 2017).

Ecotourism is a type of responsible tourism to attractive areas with natural and cultural resources, to conserve the environment and derive economic benefits for the local community (Adedeji et al., 2014; Hypolite et al., 2002; Wickramasinghe, 2013) Three sustainable goals make ecotourism lie in the sustainable development framework (Osman et al., 2012). The first goal is social and cultural development, indicated by the community participation and benefits, which can be provided by the cultural ecosystem services through opportunities for ecotourism. outdoor recreation, agricultural practices, and collective landscape stewardship (Plieninger et al., 2015). The second goal is economic by creating jobs for locals and income-

generating activities. And the third and most important goal in this study is environmental and is manifested by the conservation and valorization of natural and cultural resources, and preservation of landscapes (Açıksöz et al., 2016).

Riparian forests in Lebanon are rich in biodiversity especially with the different altitudes the river passes through from the top of the mountains till it reaches the coast. Rivers have always been a source of attraction for the first human settlements due to the presence of water for drinking, agriculture, and energy, which leaves us with many historical monuments from different civilizations mainly like bridges, aqueducts, and watermills.

Nahr el Kalb river, one of the steepest slope riversides continuing till the coast, leaving only a small space for human practices on its sides, presents a riparian forest with vegetative layers, covered water channel along the river length, scenic landscape views, orange trees orchards, old bridges, and buildings. This natural and cultural riparian forest is in danger of disturbance due to uncontrolled constructions among green spaces.

How to prevent the disturbance of the site while aiming for ecotourism?

Ecotourism is the solution for the conservation of Lebanese riparian forest

The objective of this study is to conserve natural and cultural riparian resources of Nahr el Kalb in Lebanon, by using ecotourism as a tool for conservation when conceiving a landscape management plan.

Material and Methods

The method used in this study is first of all site visits to identify natural and cultural resources of riparian forests. The technique of mapping while walking and taking photos of the scenic views, riparian forest, river bed, historical elements, human practices, allows the assessment of the actual state of the site. Following the Knowledge Attitudes Practices (KAP) method, interviews are done with 8 locals living near the site and 2 landowners. The first set of questions is about their knowledge of the existing natural and cultural resources of Nahr el Kalb riparian forest and the ecotourism potentials of the site. The second set of questions is about their intangible and tangible social values of the existing resources, values attached to the riparian landscape of Nahr el Kalb, willingness to participate in the landscape management, and for the landowners their willingness to accept ecotourists passing through their lands. The third set of questions is about the activities practiced on the site and propositions for the landscape management plan for ecotourism. The results of the site visits and the interviews are organized and analyzed following the Strengths Weaknesses Opportunities Threats (SWOT) method (Açıksöz et al., 2016; Kusumoarto et al., 2017; Osman et al., 2012; Wickramasinghe, 2013), allowing the identification of ecotourism potential elements of Nahr el Kalb. Finally, a landscape master plan of the riparian forest is created with propositions for ecotourism activities explained with sketches.

Results and Discussion

After site visits, natural and cultural resources were mapped using the technique of walking and mapping. Natural resources are the scenic views of the river and its surroundings, including the attractive steep slope riversides, with dense layered vegetation seen from the riverbeds. Nahr el Kalb riversides allowed the presence of different kinds of vegetation that are not only limited to riparian forest species like giant reed *Arundo donax*, Oriental plane *Platanus orientalis*, weeping willow *Salix babylonica pendula*, and English ivy *Hedera helix*, but also covers species that are found on higher elevations like brant's oak *Quercus brantii* and gall oak *Quercus infectoria* and brutian pine *Pinus brutia*, and that are trees native to Lebanon. Cultural resources are the water channel along the river length, which is covered by

the cement allowing people to use it as a walking path. Four old bridges, one of them destroyed, an old abandoned house, and an old small hydroelectric factory located on the path. Patches of agricultural lands, mainly of orange trees and banana plants are found next to the river, separated by fences made from the *Arundo donax* canes. This concludes that *Arundo donax* and *Platanus orientalis* are the most abundant vegetations found on the site.

Interviews results showed that 80% of the locals knew about the existing natural and cultural resources, they were able to identify the cultural resources more easily than the natural ones, and they listed them as follows: old bridges, inactive hydroelectric factory, the old house, water channel, agricultural practices, riparian forest. The reason behind not easily listing the natural resources is mainly that locals are taking the riparian forest as something granted. After explaining to them the concept of ecotourism and its three goals (Açıksöz et al., 2016), interviewees agreed on the fact that the above resources can be considered as ecotourism potential elements being unique to Nahr el Kalb river.

The intangible social values offered by the natural and cultural resources are relaxation, recreation, well-being, and memories, while the tangible values are the orange and banana picking and the giant reed canes used for fences and support for seasonal planting. Aesthetic (beauty and uniqueness of the site), cultural (memories of the active hydroelectric factory and well being), and economical (orange fruits and banana picking and selling) values are for the participants attached to the riparian landscape of Nahr el Kalb. All of the interviewees showed willingness in participating in the landscape management plan and execution and the landowners accepted the fact of ecotourists passing through their lands.

Based on their answers, walking and agricultural practices are the two activities done on the site. Interviewees proposed that ecotourists can do a forest walk, take photos, sightsee, enjoy the scenic views, visit the old buildings, help landowners with orange and banana picking, buy local products like orange juice prepared by the local women and handcrafted cane baskets made using giant reed canes.

Table 1 organizes the results collected from the site visits and the interview results, showing opportunities and threats of the natural and cultural resources and consisting of a base for the landscape management plan. Based on these findings, there is a need for weed clearing of the walking path, renovating the covered water channel and old buildings, and training guides to accompany ecotourists during their visit to make sure everything is fine. The old house will be transformed into a guesthouse where ecotourists can sleep in, and the old hydroelectric factory into a riparian forest-based museum with information about the existing trees and plants, old photos of the site, information about the planted orange trees… Putting informative signs along the walking trail to explain about the vegetation, bridges, buildings to ease the visit of ecotourists and make it beneficial. An annual orange festival can be created during the picking season in November where ecotourists help the landowners with orange and banana picking and where local women can sell their home-made products from oranges like juices, dried fruits… and cane baskets made using giant reed canes.

So the potential ecotourism elements are a walking trail along the river, locations with scenic views, a guesthouse and a museum, agricultural lands that are also a place for an annual orange festival. All these elements are mapped in Map 2. Ecotourism elements are shown in Fig. 1, 2, and 3.

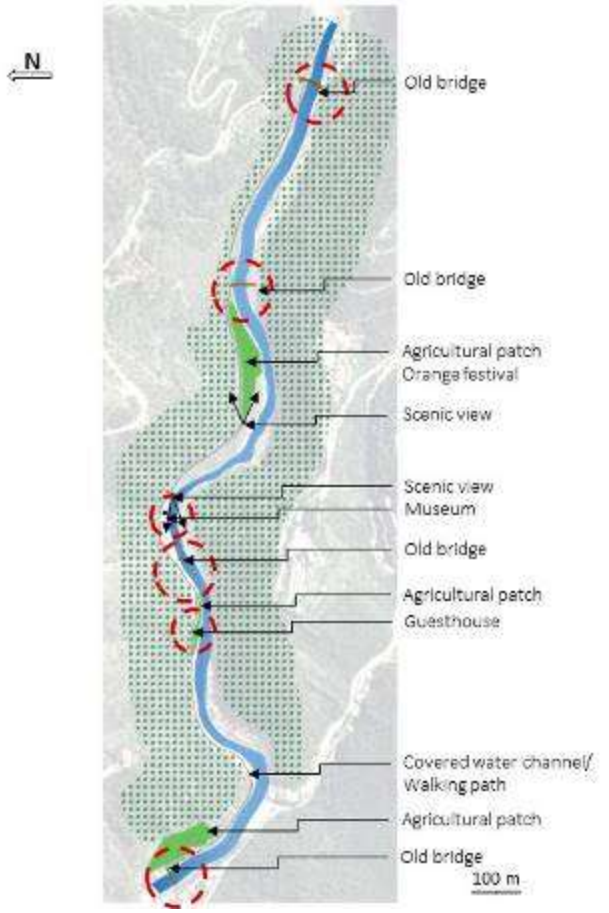
Table 1. SWOT analysis of natural and cultural resources of Nahr el Kalb riparian forest

Natural/ Cultural Resources	Strengths	Weaknesses	Opportunities	Threats
Steep slopes and layered vegetation	-scenic views -green landscape	-inaccessible for the ecotourist	-provide shade -climate suitable for walking	-wild herbs invading the covered water channel
Covered water channel	-elongates the river -water flows all year long	-very old and cracked in some places	-can be used as a walking path -provides breezy climate	-risk of destruction in the long term
Old bridges and buildings	-very attractive, built with stones -each having its own story	-abandoned buildings	-landowners agreed on opening the buildings for tourists -guesthouse and museum	-risky for ecotourists to enter the buildings
Agricultural patches	-variety of orange trees and banana plants	-lands are accessible from the walking path	-ecotourists helping during picking season -possibility of creating an annual orange festival	-risk of irresponsible behaviors and damages to the trees

*Source: Author's elaboration based on the site visits and interviews results.



Map 1. Location of the site in Lebanon



Map 2. Landscape master plan of the site showing the potential ecotourism elements

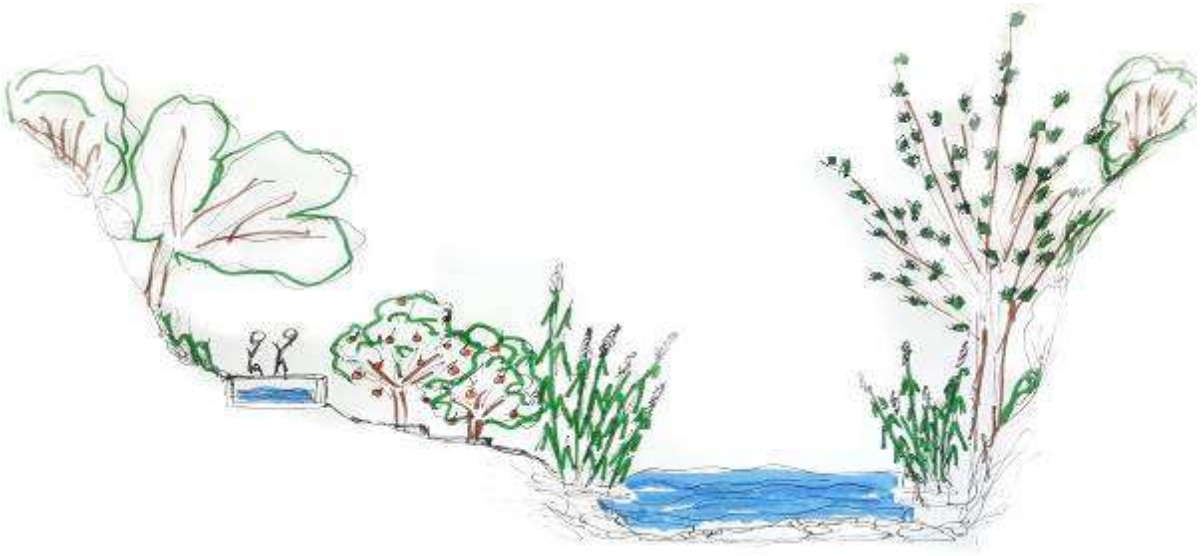


Fig.1 Transversal section of the riparian forest, covered water channel, orange trees orchards, river with the abundant *Arundo donax* and riversides steep slopes

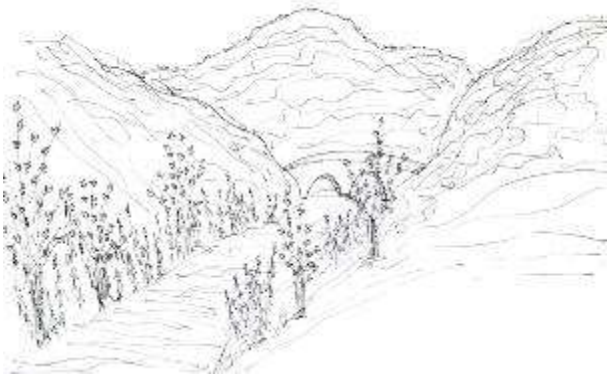


Fig.2 Scenic view from the walking path

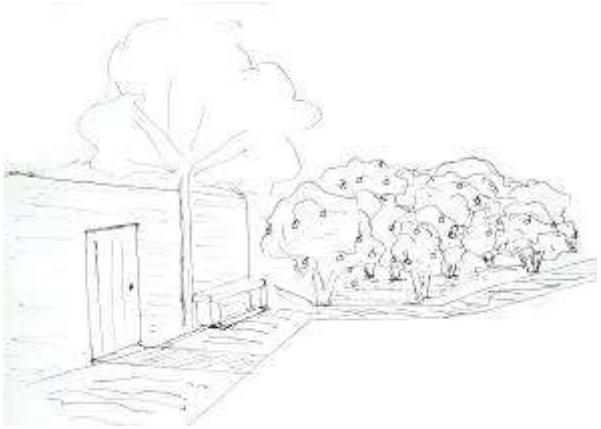


Fig.3 The old house transformed into a guesthouse and the water channel used for irrigation

Conclusion

Riparian forests are of high importance in Lebanon. Unfortunately, uncontrolled constructions form a risk for these forests, specifically Nahr el Kalb river which still preserves a natural section. Site visits and interviews with locals resulted in finding that there is a diversity in the natural layered riparian forest with scenic landscape views, and a richness of cultural resources like the covered water channel along the river, agricultural lands with orange trees and banana plants, the old house, old inactive hydroelectric factory. Locals are interested in creating ecotourism along Nahr el Kalb river and are willing to participate in the management process. Propositions like using the water channel as a walking path, with weed clearing maintenance, renovating the old buildings and transforming them into a guesthouse and a forest-based museum, using informative signs with information about the bridges, existing vegetation, scenic views, agricultural lands. Training local guides to accompany ecotourists, creating income-generating activities, and organizing an annual festival during the orange season in November where eco-tourists can participate in orange and banana picking and buy

home-made products prepared by local women. Ecotourism has a major and sustainable role in the conservation of the natural and cultural riparian forest resources in Lebanon. It should be taken into consideration in the landscape management plan of these forests.

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PSEUDOTSUGA MENZIESII (MIRB.) FRANCO IN THE NW OF IBERIAN PENINSULA: SITE QUALITY AND FORESTRY

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Abstract

Pseudotsuga menziesii (Mirb.) Franco, Douglas-fir, is a conifer of the *Pinaceae* family. The Oregon pine name, used to refer to both the tree and its wood, can take to confusion, because its needle-like leaves are not sheathed. It can reach up to 100 meters height in the country of origin –discovered by Menzies in Vancouver in 1792–, existing in Europe trees of 50 m. The cup is conical and pointed, and it needs a long time to crown, which indicates a great capacity to maintain continued growth.

The first plantations in Spain were ornamental, and in Galicia large specimens dating from the nineteenth century have been conserved. Its use during the repopulation period of the Forest Heritage was scarce, especially in the NW of Iberian Peninsula, where the oldest stands are not older than 40 years. In other Spanish regions, Forest Administration used this species more amply. According to the latest data, an area greater than 30,000 ha is estimated.

Douglas-fir grows on a wide climatic range, from oceanic to continental, with uniform distribution of rainfall. Optimal conditions for increased production are a wet oceanic climate with 2-3 months of drought. Oregon pine is a fairly demanding tree in edaphic conditions, preferring deep soils with a light texture and fertile. It is a species capable of producing high quality wood and forest treatments should be directed for this. It will be basic to prune so wood is formed free of knots and to eliminate unnecessary branches. In Galicia the maximum production range would go from 14 to 23 $m^3ha^{-1}year^{-1}$.

Key words: *Pinaceae, Douglas-fir, Site, Forestry.*

Introduction

Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, is a large conifer from North America that was brought to Europe by David Douglas in the nineteenth century. In western North America, it covers an area of almost 20 million hectares across a huge climatic range that generally features rather dry summers. Even though it was initially planted as an ornamental tree it became an important economic species because of its fast growth rate and good quality timber. Now is the non-native tree species most abundant fostered in the Central Europe (Figure 1) (Da Ronch *et al*., 2016).

Oregon pine is a large evergreen coniferous up to 60-80 m tall and with a trunk of up to 2 m in diameter (Praciak *et al*., 2013). Their needle-like leaves are not sheathed, look more like firs and spruces, and are 2-3.5 cm long. They are dark-bluish green colour above and with two white-green stomatal bands below (Eckenwalder, 2009).

In Europe, Oregon pine is one of the fastest growing trees, succeeding on a wide range of soils, best when deep, moist, well-drained, at mid-elevations and with an annual rainfall over 800 mm. It shows a noticeable soil-acidifying ability (Eckhart *et al*., 2019).

Also, the initial plantations in Spain were ornamental, and in Galicia big specimens dating from the nineteenth century have been conserved. Its use during the afforestation period of the Forest Heritage was scarce, especially in the north-western, where the oldest stands are not

older than 40 years. In other regions, Forest Administration used this species more amply (La Rioja, País Vasco and Navarra).



Figure 1. Regions of Europe (*black triangles*) where there has been significant afforestation through *Pseudotsuga menziesii* (Source: Isaac-Renton *et al*., 2014).

According to the latest data, Douglas-fir covers more than 800,000 ha in Europe, of which 50% are in France, 25% in Germany, and the remaining 25% are distributed across other European countries (Eckhart *et al.*, 2019). Currently, an area superior than 30,000 ha is estimated in Spain (Solla *et al.*, 2006).

High growth ratios, high reproduction aptitude, great acclimatization, excellent wood properties, and a low number of pests and diseases are some factors that have contributed to its success and spread in Europe, performing much better than Norway spruce (*Picea abies* (L.) Karst.) on similar site quality (Schmid *et al.*, 2014). Most of the Galician current stands are in small forests on agricultural land and privately owned, with growth exceeding 19 $m^3ha^{-1}year^{-1}$ (Figure 2). It will be fundamental to prune so wood is formed free of knots and to eliminate excessive branches. For all this, *Pseudotsuga menziesii* is widely regarded as an especially promising option to increase productivity and to adapt European forests to climate change scenario (Spiecker *et al*. 2019).

Together with the increasing promotion of Douglas-fir, concerns about possible negative ecological impacts are also showed from the side of the sustainable management. Researches of ecological consequences of this species cultivation in Europe were reviewed by Schmid *et al*. (2014). This analysis concludes that in Europe, it regenerates naturally mainly on poor sites (dry and acidic), where it does not compete with native tree species, and the ecological impacts to be minor compared to other non-native trees. Nevertheless, Douglas-fir could cause changes in species composition.

The objectives of this study were: i) to determine appropriate seed origin for repopulation and the site of optimum quality for the species; ii) to show the effect of the different forestry treatments on productivity of Oregon pine in Galicia, north- western of the Iberian Peninsula.

Material and Methods

Study area. Galicia Forest Plan, 1992

The study area includes the Autonomous Community of Galicia, located in the north-western of Spain, with an area of ~ 3 million hectares. The average altitude is 508 m and slopes of more than 20% are present in half the land. The lithological composition is heterogeneous,

although siliceous substrates predominate. The climate is diverse and is generally classified as wet oceanic with some Mediterranean influence in some areas. The annual precipitation varies between 600 and more than 3000 mm (Diaz-Maroto *et al.* 2006).

Galicia Forest Plan, elaborated in 1992 and revised in 2018, sets out a number of forest regions that constitute the basic classification to establish index species for afforestation. With an execution period of 40 years, the Plan included the repopulation of 70,234 ha of Oregon pine, most of which would be located in the provinces of Lugo (34,179 ha) and Ourense (22,631 ha). The regions in which the Plan considers as index species to *Pseudotsuga menziesii* are the Central Plateau, the Northeast Mountain and Southeast Mountain. In the remaining regions, the increased presence of *Eucalyptus globulus* Labill., *Pinus radiata* D. Don. or *Pinus pinaster* Ait., makes Douglas-fir is less represented.

Interest use in afforestation is due to the possibility of producing higher quality wood than with other conifers currently used commercially in Spain. Performance is excellent with a logging between 50 and 70 years, and its requirement less than most hardwoods. Tolerance in mixed stands and adaptability to different conditions of altitude, exposure and temperatures allow you to be valid in a part of the pine own sites, without forgetting its ornamental, landscape and recreational importance (Praciak *et al.*, 2013).



Figure 2. Oregon pine (Monte Rioboo, Lugo). Average growth ~ 15 $m^3ha^{-1}year^{-1}$.

Results and Discussion

Seed origin of afforestation and the site optimal characteristics

Now, Oregon pine planting is often discussed in relation to the predictable adaptations of forest management to current scenario of climate change. With climate change states expecting higher mean annual temperatures and increased summer drought, it may be as an alternative tree species even in those countries where the wood market for this tree species is not yet established (Hasenauer *et al.*, 2016; Eckhart *et al.*, 2019). For Douglas-fir to be introduced in new areas adequate seed material is essential. Thus, in both France and Germany, around eight million seedlings are needed each year for afforestation. In recent times, huge efforts were made to establish a basis for suitable reproductive material production of this species (Spiecker *et al.*, 2019). The difference in the behaviour of different origins of the seeds is such that it could determine the success or failure of the repopulation.

The tests that the Research Centre of Lourizán (Pontevedra, Spain) has been doing for almost 40 years have allowed us to know the most adapted American provenances for reforestation in

the North of Spain. The Department of Agriculture of the United States has made a division into geographic regions as distinct provenances, and which are also referred to as seed areas (Solla *et al*., 2006).

The Oregon pine seedlings normally used in afforestation grows in nursery both bare-roots as container. In the second case, it has a root that develops in the container and the end of the culture may be extracted with the plant root ball, so set of root system with the substrate has been developed.

The area increase covered by Douglas-fir, by both natural regeneration and afforestation, and its possible adverse effects on biodiversity and habitats are both associated with another important topic: the invasiveness of this species. In some European countries Douglas-fir is considered to be invasive, whereas in others it is not. Nevertheless, it should be highlighted that, despite the fact that Douglas-fir was shown to have an effect on forest species composition and habitats, none of the researches conducted argue for an absolute prohibition (Schmid *et al*. 2014).

The extent of the natural area of the species results in very different ecological sites, making it necessary to define different regions of origin. In this way, we can choose among provenances with better resistance to spring frost because to sprout late or sources that best support the summer drought. However, they also be could establish general characteristics of the species to determine suitable sites for repopulation (Spiecker et al., 2019).

Forestry treatments to improve the productivity of Oregon pine in Galicia

As I mentioned above, one of the exceptional characteristics of Douglas-fir is the species' great growth potential. In central Europe, Douglas-fir clearly is among the most productive conifers. For example, in France Douglas-fir now covers slightly under 400,000 hectares with an annual grow of 14.8 $m^3.ha^{-1}$. In Germany, according to the latest national forest inventory (2013), the species develops across more than 200,000 hectares and shows an average annual grow of 18.9 $m^3.ha^{-1}$. In both countries, Douglas-fir growth exceeds the average annual increment of the conifers by 76% (France 8.4 $m^3.ha^{-1}$) or 47% (Germany 12.8 $m^3.ha^{-1}$), respectively. Among the major conifers Douglas-fir is the fastest grower, overtaking even Norway spruce (France 13.2 $m^3.ha^{-1}$; Germany 15.3 $m^3.ha^{-1}$) (Kohnle *et al*., 2019). However, despite these spectacular growths, in Galicia the maximum production range would go from 14 to 23 $m^3ha^{-1}year^{-1}$ (López-Sánchez and Rodriguez-Soalleiro, 2009).

So to achievement successfully the significant growth potential of Douglas-fir, management has to address the following aspects (Kohnle *et al*., 2019):

- Definition of the production aims to be reached (e.g. timber dimension, quality and different uses).
- Establishment of site-adjusted, healthy and vigorously growing stands.
- Forestry treatments (e.g., spacing, thinning, pruning, even or uneven structure) that achieve an optimum between the characteristics of (diameter) growth speed, quality development and associated potential risks (e.g. storm damage).
- Control/maintenance of desired admixed species.

In many *Pseudotsuga menziesii* stands in the Northwest of Spain there are almost total absence of cultural treatments, such as clearing, pruning and thinning. In these cases, the density of trees is very high. There are many dominated trees and some already dead, which is a source of pest proliferation. If not pruned, Oregon pines have branches from the base, which will have abundant knots and lose quality. Oregon pine is a species capable of producing high quality wood. Therefore, applied forestry must be guided to obtain that product. To achieve this, it will be necessary to prune, so that wood is formed free of knots, and make thinning, to

remove branchy trees, forked or low vigour, concentrating all the productive capacity of the forest in the best trees (López-Sánchez and Rodriguez-Soalleiro, 2009).

Conclusions

In North America, Douglas-fir covers an enormous area across a huge climatic range that generally features rather dry summers. In Europe, it was introduced as an ornamental tree in arboretum and parks. From the end of the 19th century it was planted at a progressive rate in the forests of various European countries. This has led to the current high presence of trees up to an age of 60 years. Today Douglas-fir is the second most common non-native tree species in European forests where it covers more than 800,000 hectares. The largest area of Douglas-fir is found in France, followed by Germany where it has rapidly become the most widespread non-native tree species. In other European countries, Douglas-fir is still growing to a considerable extent, e.g. in Spain there is 30,000 hectares of afforestation. The main reason for growing Douglas-fir is its high productivity and the excellent wood properties. The value chain of Douglas-fir provides thousands of jobs and tens of millions of euros worth of income. Today, other factors have to be considered as well. These may be positive, such as Douglas-fir's capacity to adapt and mitigate to climate change, or negative, such as public perceptions concerning detrimental effects on native ecosystems and their biodiversity.

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PLANT DIVERSITY IN TREE STRATA AND ITS RELATION TO SOME FEATURES OF BEECH STAND IN HYRCANIAN FORESTS

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Abstract

For this research, field data from 24 circular 400 m^2 sample plots in the inventory grid of 100 m × 150 m were used in beech stand, northern Iran. For characterizing tree-layer diversity, tree- layer species richness (SR), Shannon–Wiener diversity index and Hill's evenness index were used. Diameter and height of beech trees were measured per sample plot. Herb-layer species richness (herb-layer SR) was estimated in each plot. Litter and humus layer thickness were measured at center and 4 corners of sampling points. Result showed that a total of 7 tree species including *Fagus orientalis*, *Carpinus betulus*, *Alnus subcordata*, *Tilia begonifolia*, *Acer velutinum*, *Acer cappadocicum* and *Prunus avium* were recognized in 24 plots. Totally, 23 herbaceous plants belonging to 21 families were found distributing in different plots within forest which higher life form was presented by Hemicryptophytes. There was a significant difference between dominant and codominant layers regarding to tree species richness (SR) and Shanon-weaver diversity index ($P<0.01$), and higher mean values belonging to dominant layer. The herb-layer richness correlated significantly with litter thickness, whereas there was no correlation between other factors. SR of tree layer was negatively and significantly correlated with DBH and height of beech trees and Shanon's diversity and Hill's evenness indices of tree layer were negatively correlated with beech frequency.

Keywords: *Tree strata, Diversity, Beech forest, Herb-layer, Hyrcanian region*.

Introduction

One parameter that characterizes forest ecosystems as stable and efficient is diversity, therefore biodiversity maintenance is important for sustainable forest management (Polyakov *et al.*,2008). Identifying the interactions between management methods and the forest vegetation contributes to the development of more sustainable forest management methods (Durak, 2012). Intact forests are quite helpful in studying vegetation structure and dynamics (Vacik *et al.*, 2009). Forest ground vegetation comprises the largest number of plant species that take part in forest dynamics (Khanina *et al.*,2007).Vertical forest structure is an interesting characteristic of forests that appeals to many disciplines and is a constant topic of discussion in ecosystem management. The vertical forest strata of tree crowns can manipulate both tree growth and understory community structure (Latham *et al.*,1998).

Since trees provide habitat and a diverse range of resources for a vast array of plants and animals, tree diversity is central to total forest biodiversity (Ozcelik, 2009). Due to widespread ecological impacts of trees , plant biodiversity are believed to be affected by tree species identity and composition (Barbier *et al.*,2008) and consequently sustainable forest management has always seek to preserve species diversity in managed forests (Ito *et al.*,2006). According to Potts *et al.* (2005), incorporating preservation into sustainable forestry calls for the precise estimation of pre-harvesting biodiversity and the observation of tree diversity. As Motz *et al.* (2010) asserted, forest structure is a fundamental aspect of forest ecosystem management. Individual tree species in forest communities tend to be spatially

related to one another and any changes in this spatial structure occurs over long period of time (Shimatani and Kubota, 2004).

Hyrcania (Caspian) forests are positioned at green strip stretching over the northern slopes of Alborz range and southern coasts of the Caspian sea (Haghdoost *et al*., 2011). The vegetation is consisted typically of deciduous broad-leaved forests (Akhani *et al.,* 2010). Pure and mixed beech (*F.orientalis*) forests are the most important components of this ecosystem, making up the richest and the most beautiful forests of Iran (Salehi Shanjani *et al*., 2011). Nouri *et al*.(2011) reported that in a multilayer forest in Hyrcanian region, the richness index showed diminishing trend from the third layer to first one and the heterogeneity indices increased from the third layer to second and then decreased to first layer. The present research aims at answering the following questions:

1. Is there any difference between different tree layers regarding to species diversity indices?
2. Is there any relationship between tree layers diversity and herb-layer richness, height, diameter and crown depth of beech as well humus thickness and litter depth?

Material and methods

Study area

The study was conducted at Azandeh districts(53̊ 02'-53̊ 07'E,36̊ 04' -36̊ 09' N) in forestry plan of Tajan watershed, located in Mazandaran,northern Iran. The selected compartment (65.6 ha) was positioned at western aspect . The topography is characterized by slopes about 30% at elevations of 1150-1440$_m$ a.s.l.The mean annual precipitation is 562.4mm. The mean annual air temperature in the region is 7.4–22.3 ◦C, The region has a humid temperate climate, and dominant type of soil is forest brown soil.*Fagus orientalis* is the dominant tree species in this site with *Carpinus betulus,Alnus subcordata,Acer velutinum, Acer cappaducicum* and *Prunus avium*.

Data collection

For this study, field data from 24 circular 400 m^2(radius = 11.28 m) sample plots (Gilliam 2002;Destan *et al*.2013; Bartels & Chen 2013) in the inventory grid of 100 m × 150 m were used.Selection criteria were tree-species richness and tree-layer composition.Only trees with a diameter at breast height (dbh) of at least 2 cm were considered.For characterizing tree-layer diversity,tree-layer species richness(tree-layer SR),Shannon–Wiener diversity index H'[H' = -

$Pi\ (lnpi)$, and Hill's evenness index($E = (1/\lambda - 1)\ /(e^{H'} - 1)$)

where P_i=proportion of individuals in *i*th species ,λ=Simpson's diversity index and *H'* = Shannon's index were used.Diameter and height of beech trees were measured per sample plot.Herb-layer species richness (herb-layer SR) was estimated in each plot. Understorey vegetation surveys were conducted during the period of peak vegetation cover (between Jul and Aug 2013) and when all deciduous tree species had leafed. Litter and humus layer thickness were measured by ruler at center and 4 corners of sampling points. Samplings were done for each plot in summer 2013.

Statistical analysis

To analyze difference between tree layer biodiversity indices, two independent samples T test was used. Pearson's rank correlation was applied to analysis relationships between species diversity and the measured environmental variables. All statistical analyses were used the statistical package SPSS version 18. The level of significance of statistic tests were as follows: $p< 0.05$; $p<0.01$.

Results

A total of 7 tree species including *Fagus orientalis,Carpinus betulus,Alnus subcordata,Tilia begonifolia,Acer velutinum,Acer cappadocicum* and *Prunus avium* were recorded in 24 plots.Totally,23 herbaceous plants belonging to 21 families were found distributing in different plots within forest which higher life form was presented by Hemicryptophytes (Table1). There was a significant difference between dominant and codmoinant layers regarding to tree species richness (SR) and Shanon-weaver diversity index (*P<0.01*), and higher mean values belonging to dominant layer (Table 2,3). The herbaceous richness correlated significantly with litter thickness (Table 4),where as there was no correlation between other factors (Table 4). SR of tree layer was negatively and significantly correlated with DBH and Height of beech trees and Shanon's diversity and Hill's evenness indices of tree layer were negatively correlated with beech frequency (Table 5).

Table1- List of plant species in beech forest

Species	Family	Life form
Euphorbia amygdaloides L.	Euphorbiaceae	Ch
Phylitis scolopendrium (L.) Newn.	Aspleniaceae	Ge
Polystichum aculeatum (L.) Roth	Aspidiaceae	Ge
Carex sylvatica Huds	Cyperaceae	He
Sanicula europaea L.	Umbelliferae	He
Mercurialis perennis L.	Euphorbiaceae	He
Dryoptris filix-mas (L.) Schott.	Aspidiaceae	Ge
Galium odoratum (L.) (Scope.)	Rubiaceae	He
Viola alba Bess.	Violaceae	He
Brachypodium sylvaticum (Huds.) P. Beauv.	Graminae	He
Lamium album L.	Labiatae	He
Primula heterochroma Stapf.	Primulaceae	He
Cephalenthera longifolia (L.) Fritsch	Orchidaceae	Ge
Solanum dulcamara L.	Solanaceae	He
Polygonatum orientale Desf.	Liliaceae	Ge
Cyclamen coum Mill.	Primulaceae	Ge
Hypericum androsaemum L.	Cyperaceae	Ch
Ruscus hyrcanus Woron.	Hypericaceae	Ph
Rubus hyrcanus Juz.	Rosaceae	Ph

Oplismenus undulatifolius (Ard.) L. P. Beauv.	Graminae	Th
Circaea lutetiana L.	Onagraceae	He
Pteris cretica L.	Pteridaceae	Ge
Lathyrus Laevigatus(Jacq.)Grake.	Papilionaceae	Th

Ph:Phanerophyte, Th:Therophyte, Ge:Geophyte, Ch:Chyrptophyte, He:Hemichryptophyte

Table 2.Two independent samples T test comparison of Biodiversity indices for different tree layers

Index	F	df
SR	1.381**	46
Shanon-weaver(Diversity)	0.527**	46
Hill(Evenness)	0.062	46

**: Significant at the 0.01 level.

Table 3. Mean values (±SE) for biodiversity indices in different tree layers

Index	Dominant	Codominant
SR	**3.25±0.193**	1.96±0.195
Shanon-weaver(Diversity)	**1.340±0.067**	0.88±0.84
Hill(Evenness)	0.85±0.081	0.78±0.063

Table 4. Correlation of herb - tree layer species richness and some factors in forest

SR	Lither(cm)	Humus thickness(cm)	Beech(%)
Herb layer	r=0.424*	r=0.178	r=0.274
	P=0.044	P=0.417	P=0.196
Tree layer	r=-0.0513	r=-0.067	r=0.08
	P=0.806	P=0.755	P=0.710

*Significant at the 0.05 level.

Table 5. Correlation of tree layer species diversity indices and some features of beech trees (*Fagus orientalis*) in forest

Index	Beech(%)	Crown	DBH(cm) depth(m)
SR	r=-0.375	r=-0.110	r= **-0.492****
	P=0.071	P=0.608	P=0.015
Shanon-weaver diversity	r= **-0.628****	r=-0.076	r=-0.331
	P=0.001	P=0.723	P=0.114
Hill	r= **-0.661****	r=0.071	r=0.086
	P=0.739	P=0.189	P=0.002

,**Significant at the 0.05 , 0.01 level.

Discussion

Nagaike *et al.* (2012) refer to the effect that forest management practices can have on plant species diversity as a significant factor which aids in ecologically sustainable forest management. Vockenhuber *et al.* (2011) also stated that herb layer diversity is influenced by tree layer diversity when the environmental conditions available to herb layer plants are modified. The present study revealed that stand structure had influence on tree diversity and Species richness (SR) in forest. Tree species richness was relatively higher in higher classes as well as those with larger diameter. This finding suggests that the more developed the stand structure are, the more tree species are present in a stand. Although forest management has the potential to influence the composition of the tree layer, inter-specific competition may decrease the species number in a forest stand (Neumann and Starlinger, 2001). One important components of biologically spatial structure is the species composition (Huang *et al.*,2003). A mixed-species forest is the one with at least seven tree species of 1–10 individuals in the whole forest plots. In the present study, most individual stems belonged to beech trees. The results indicated that beech trees with higher frequency would influence species diversity. The presence of seedlings of beech species revealed that they were regenerating adequately in all stands, despite the competition from the sub-canopy and herbaceous species. All plots had a highly heterogeneous distribution of trees and could be considered as one of the high diverse forest. The undisturbed stand had a high density of tree species due to restricted access of humans. There was a significant correlation between herb-layer SR and litter layer thickness. Many authors have regarded litter layer thickness as a factor influencing herb-layer density and composition (Molder *et al.*, 2008). Ground flora species have varying degree of litter penetration (Sydes *et al.*, 1981).The amount of soil litter depends on the balance between litter production and litter decomposition (Barbier *et al.*,2008). Decomposition rate of litter is under the influence of the structure of the organic surface layer (Jacob *et al.*,2010). The thicker organic surface layer in the stand dominated by *F. orientalis* might show high amount of litter. Moelder *et al.* (2008) also stated that beech foliage decomposes poorly, which results in thick litter layers. Being an important ecosystem factor, litter quality can control undergrowth species richness not only through decomposition of organic layers directly, but also through longer-term changes in soil pH and moisture (Kooijman and Cammeraat, 2009). In some plots, the higher leaf litter layer could impede light transmittance and represent an obstacle for the growth of herb plants. As a result, values of SR tend to decrease when leaf litter cover is high. In the region, over- storey broad-leaf composition had no correlation with herb layer richness (SR). According to Moelder *et al.* (2008), similar results can be found concerning diversity relationship between tree layer and herb layer. Higher SR of herb layer, comparing with over-storey might be due to the wetter condition provided by over-storey canopy which allows plants to be more shade tolerant in beech forest in temperate region. North *et al.* (2005) referred to the existing soil moisture as the major influence on understory plant composition. Diameter and height of beech trees decreases with an increase in SR of trees in over-storey broad-leaf composition which it could be attributed to compete for light and nutrients. The supply conditions in the forest under-storey can be controlled by species composition of the over-storey tree layer (Bartels and Chen, 2013).The correlation between tree- layer diversity and beech proportion in tree layer (negative correlation) were significant that is inconsistent with the study carried out by Moelder *et al.* (2008) in which sites were described as a gradient from pure beech stands to mixed stands deciduous tree species. Lower beech proportion had a more profound effect on herbaceous under-storey diversity compared to the number of secondary tree species such as *Carpinus betulus*, *Alunus subcordata* and *Acer velutinum* in site. Understanding

herb and tree layers species diversity and distribution patterns is important for forest managers to evaluate the complexity and resources of these forests.

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PRACTICE OF TRADITIONAL CROP PRODUCTION IN THE NATURAL FORESTS OF DRYLAND OF SUDAN IMPROVED THEIR NATURAL REGENERATION

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Abstract

The survival of natural forests in Sudan is facing a great challenge due to the conflict to secure food for ever increasing population on the account of their diminishing stocked areas and the efforts to manage them to sustain their environmental and economic values and services that are hardly considered by the politician and strategic planners. Each argument is striving to scrub out the other without providing evidence denying the achievement of both simultaneously. Therefore, this study investigated the effect of traditional cropping practices by the local farmers inside the natural forests on their natural regeneration stocking density and performance. The natural regeneration was systematically sampled in four compartments in natural forests of *Acacia seyal*. One was left uncultivated after felling and the other three were cultivated for consecutive two, three or four years. Inventory of natural regeneration was carried out accordingly. Significant regeneration was found surviving on all compartments but it was denser where it succeeded cultivation. It was 750 seedlings ha^{-1} in uncultivated compartment and 1000 – 4000 seedlings ha^{-1} in cultivated ones. Moreover, the regeneration was vigorous and taller following the cultivation. The results underlined the oversight understanding of the negative impact of cropping on regeneration of natural forests and favored its integration in the restocking programs. This could solve the conflict on land and encourage the participation of the local communities in the management of forests as well as contributing in food security.

Keywords: *Acacia seyal, traditional cropping, practices, regeneration, integration*

Introduction

In the traditional agricultural practice the farmer clears small area and cultivates it for successive four years before abandoning it to adjacent one which is naturally stocked with trees (Mustafa, 1997).Suleiman (2014) studied the vegetation regrowth on abandoned agricultural land in Gadaref region of Sudan by investigating the impacts of the previous cultivation period and the duration of the fallow on the subsequent natural regeneration in terms of vegetation composition, structure and diversity. The results regarding the regeneration showed that there was a general tendency to decreasing with increasing in the period of cultivation and the capacity might not be sufficient to reach the climax vegetation. Other study carried out on the effect of past and current land use, specifically clear felling, on vegetation structures as well as recruitments, found that they were negatively affected by both (Marinho *et. al.,* 2016). Mukul *et al.* (2016) found significantly recovery of aboveground biomass along a fallow gradient in post shifting cultivation secondary forests in an upland area in the Philippines. Investigation of the effect of past agricultural use on regeneration of Brazil nut found in cultivation fallows was studied by Paiva *et al.* (2011) and they found that the species resprout successive with increase in the fallow periods of slash and burn cycles.
Mechanized agriculture that deemed at the initial the promising increase in agricultural production proved that is unsustainable and soon so more deforestation is needed to

compensate for the productivity declining. This may underline the justification of the mechanized monoculture approach and favor the traditional agricultural practice that pursued by the local communities in the region long time ago, but at time being it is not permissible for the reason that there is no land, because either taken for mechanized agriculture or gazette reserved forests.

Foresters are still perceiving shifting cultivation as deleterious and not allowed to be practiced in the reserved forests, although they are not yet proficient to manage them because of meager resources and denying of the rights and privileges of the local communities. Their belief is based on assumption that agriculture is one of the practices that could deplete the soil seed bank and destroy the coming up natural regeneration. Could this absolutely valid or forged, hence, this study was investigated the effect of cropping on the natural regeneration stocking density and growth.

Material and Methods

Study was carried out in 2016 in mature natural *Acacia seyal* forest at Khor Donia in Sudan (11º49´N, 34º24´E, and altitude 470 m). The soil is very dark grayish-brown clay, slowly permeable when wet and shrinks on drying forms a network of cracks, alkaline and topography is generally flat. The average annual rainfall is about 700 mm and most of it falls in June to September.

Over 600 hectares were cleared from pure *A. seyal* in Khor Donia forest without leaving any mother trees or broadcasting seeds to regenerate the site, hence, any natural regeneration came up was apparently from the existing soil seed bank. Thereafter, the survey of natural regeneration on the site was carried out after three years. Contour map was drawn for the site at initial and accordingly the site was divided into three terrains; on-flow (where rain water collects), off-flow (where rain water runs) and intermediary surfaces. Parallel survey transects were prearranged over the whole site at 500 meter from each. Sample plots, each measuring 40x40 meter, were laid at 750 meter apart along each transect. All surviving seedlings were recorded in each plot and their diameter at ground level (d_0) was measured. The data were used for determining the density of occurrence over the site as well as the seedlings diameter distribution. Based on experience and estimates provided by Adams (1967), diameter was grouped into classes to estimate the age of the seedlings. When data collated, the T-test was operated to compare the seedlings density across the contour at three distinct locations; on-flow, off-flow and intermediary gradients.

The natural regeneration was also assessed after the leaf flushing on three flat locations in Khor Donia natural forest; all were cleared from *A. seyal* trees and cultivated with Sorghum [*Sorghum bicolor* (L.) Moench] for two, three or four successive years and then abandoned. The locations were strictly managed to avoid any transport of seeds to them. The farmers weeded all herbaceous plants during the cultivation using local traditional tools. Systematic sampling was utilized to count and measured the diameter at ground surface of the natural regeneration of *A. seyal* in plots laid along transect lines well represented the location. The diameter was grouped into classes to estimate the age of the seedling based on the methodology used by Adams (1967). When data collated, the T-test was operated to compare the seedlings density at the locations.

Results and Discussion

The density and distribution of surviving seedlings recruited for three years in the cleared natural forest site was considerably varied in association with the surface slope (Table1). The seedlings were sparsely grown on both off-flow surface, where rain water runs off, and on-

flow surface, where rain water collects. The intermediary surface, which constitutes about 80% of the area of the site, significantly ($p < 0.5$) sustained denser recruitment. The overall mean was 788 seedlings ha^{-1}. As far as the shoot height was considered, the seedlings grew on on-flow surface were significantly ($p < 0.5$) longer than those on off-flow and intermediary surfaces (Table 1). The variation in the seedlings density within the cleared forest site is merely attributed to the surface gradient. Rain water runs from off-flow surface and hence the soil moisture not maintained sufficiently long to sustain the seedling establishment. The insufficiency of the soil moisture might also have limited seeds production and consequently contributed in poor regeneration. The rain water collects on on-flow surface to retain long-lasting soil moisture that could induce germination of the seed. Moreover, the run-off might transport the seeds onto the on-flow site when temporary flooding occurred. Despite seemingly favorable regeneration conditions at on-flow site, the seedlings density was even sparse compared to the other two slopes. This may attribute to the temporary inundation that happened during the rainy season on the on-flow site. It is likely some of the seeds suffocated and also some of the emerging seedlings. The significance of inundation to preclude *A. seyal* from the vegetation structure in the depressions had been reported earlier (Buting and Lea, 1962). The intermediate surface at the site of the study constitutes about 80% of the area and it was found to sustain the dense population of seedlings (1200 ha^{-1}). This was significant as far as restocking program is considered specially the cohort-type seedlings population survived the first dry season and passed the most critical vulnerable stage in their establishment and would have good chance of surviving and developing to mature tree if not disturbed by severe drought, heavy grazing or fire. However, this regeneration capacity seems sufficient to restock *A. seyal* on abandoned or cleared sites (Freigoun, 2001; Mohammed, 2011)

The seedling diameter distribution over the whole site indicated regeneration pattern that 2% of surviving seedlings most likely regenerated during the first growing season and more than 87% of smaller seedlings regenerated during the third growing season (Fig. 1). Hence, it started very sparsely and then tended to shoot up during the third season (year). Absence of seedlings with diameter larger than three cm on the on-flow site indicated either delayed or impaired regeneration. The increase in the number of the smaller seedlings regenerated during the third season to 90% on off-flow surface compared to other ones suggested the delay of regeneration on the off-flow site in general. However, the regeneration pattern on intermediary slope resembled that on the whole site.

Regeneration was dense when succeeded shifting cultivation, it was 4011 seedlings ha^{-1} after elapsed two years from abandonment and then decreased to1078 seedlings after four years (Table 2). Since the second year from abandonment the seedlings developed stem diameter at ground surface greater than two cm on average and that increased to three cm during the succeeded two years and the shoot height showed considerable increase to 178 cm after four years from abandonment compared to 141 cm after three years (Table 2). About 80% of the seedlings were developed shoot of less than one cm diameter during the first two years from abandonment (Fig. 2). That was decreased to less than 20% during the succeeded two years. The density of the seedlings with stem diameter greater than three cm was increased from 3% in the first two years from abandonment to 43% after four years.

Table 1: Density (no.) and height (cm) of seedlings regenerated through three years from cleared and uncultivated site previously stocked with pure natural *Acacia seyal* in dryland of Sudan

Slope of site	Density/plot	Density ha^{-1}	Shoot height (cm)
	Mean ± SD		Mean ± SD
Off-flow surface	34^{b} ± 25	212	38.7^{b}±23.8
Intermediary surface	189^{a} ±75	1181	43.9^{b}±22.5
On-flow surface	28^{b} ±19	175	64.3^{a}±31.4
Over the site	126 ± 99	788	46.2±28.7

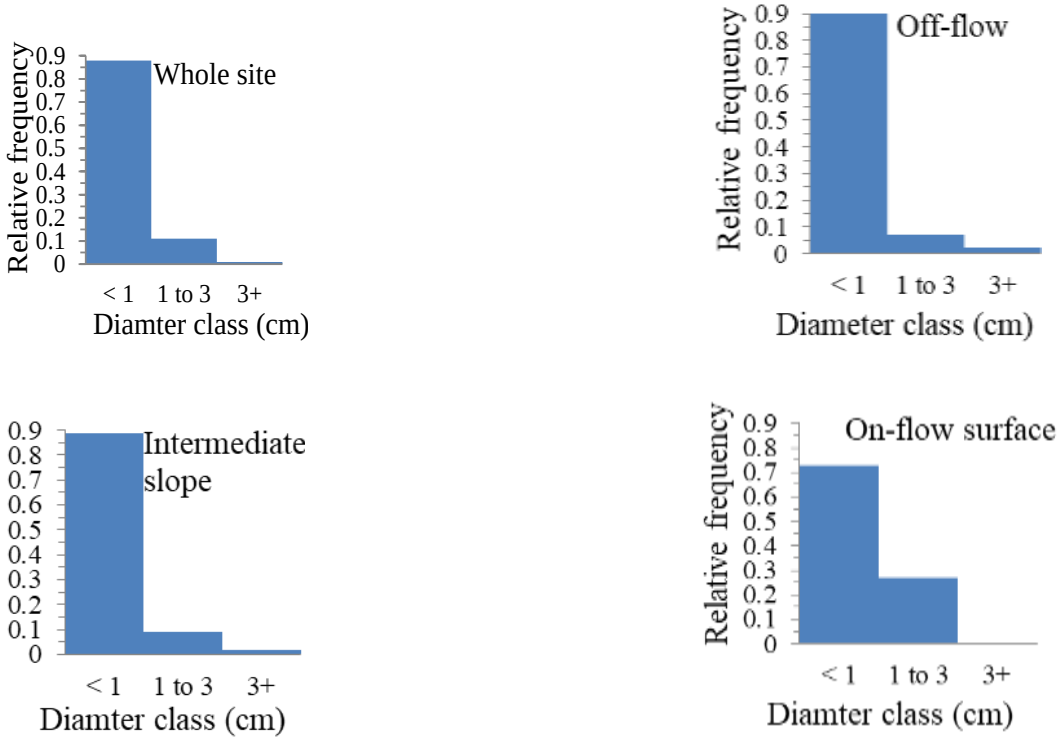


Fig. 1: Relative frequency distribution of stem diameter at ground level of seedlings regenerated for three years from clearance of pure natural *Acacia seyal* forest in dryland of Sudan

Table 2: Density and growth of natural seedlings grown on abandoned land after cleared from pure natural *Acacia seyal* and cropped with *Sorghum bicolor* under shifting cultivation (n=10)

Year elapsed[1]	d_0[2] (cm)	Height (cm)	N0. of seedlings ha^{-1}
	Mean ± SD	Mean ± SD	
2	2.1±0.93	141±58.7	4011
3	2.4±0.95	141±46.6	1956
4	3.0±1.36	178±62.5	1078

[1]Years elapsed from abandonment; [2] Diamter at ground level

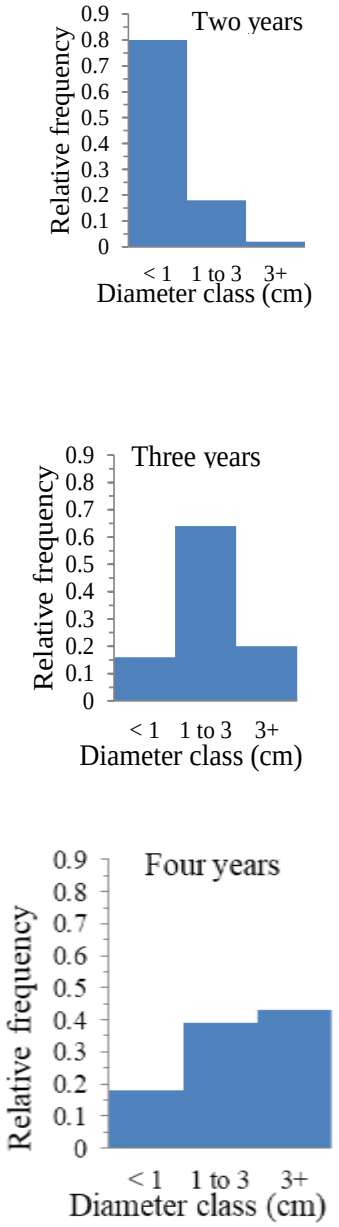


Fig. 2: Relative frequency distribution of stem diameter at ground level of natural seedlings regenerated on site cleared from natural *Acacia seyal* forest and cultivated with *Sorghum bicolor* for successive four years then abandoned. Assessment was carried out after two, three and four years from abandonment.

Conclusion

In conclusion, the significance of shifting cultivation following tree felling in enhancing the natural regeneration and growth of the seedlings of *A. seyal* is obvious and has to be considered when management programs are planned. Combined system that integrates crop cultivation and forest management is recommended to cease the irrational conflict on land resources to secure food for the increasing population and on the other hand to conserve the forests resources

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ACACIA MILLEFERA (VAHL) VS MORINGA OLIFERA LAM. AS REFORESTATION OPTIONS IN BUTANA AREA, SUDAN

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Abstract

Forest rehabilitation via reforestation with and without fencing was carried out in Butana area by plantation seedlings of *Acacia millefera* and *Moringa olifera* as options. The trial was conducted at Sobagh village Central Butana (IFAD, enclosure) and EL-Adid-ELtual village Southern Butana (farmer field without fencing).Trees measurements were done for successive two years (2013-2014) during the rainy season. Measurements included: height (cm), stem diameter (mm), number of branches per plant, Survival (%). Results showed that shoot length and stem diameter increased with plant age without significant differences among tree species, However, number of branches per plant, decreased significantly in the fifth ($p < 0.04$) and seventh months ($p < 0.03$) months for *Moringa olifera*. Survival% was better for *Acacia millefera* (56%) than *Moringa olifera* (18%), since it showed steady increase with advancement of age. Generally, trees' performance was better at Sobagh enclosure than EL-Adid ELtuwal village.

Keywords: *Reforestation, Forest, Acacia millefera, Moringa olifera, Butana area.*

Introduction

Deforestation and forest degradation are in fact the major threats to the forestry development in Sudan. The Global Forest Resource Assessments (FRA) of 1990 and 2010(FAO, 2010) indicated a declining trend in the forest cover of the ''Old Unified'' Sudan from 76.38 million ha (32.1%) to 69.95 million ha (29.4%), respectively. Deforestation and forest degradation are important contributors to global GHGs emission, but if these processes are controlled, forest can significantly contribute to climate change mitigation (IPCC, 2006). Reforestation is an effective mitigation option; it can be used to rectify the effects of deforestation. According to Hassan &Tag (2017) the definition of reforestation is: The re-growth of forests after a temporary (< 10 years) condition with less than 10% canopy cover due to human-induced or natural perturbations. Reforestation and afforestation activities have been practiced in Sudan since 1911 and restricted to areas constituted as reserves and subsequently put under management. They are exclusively owned by the Forest National Corporation (FNC). The annual FNC reforestation and afforestation programs ranged from 2.100 ha to 2.250 ha during the period 1910-1950, to some 35.000 ha during the 1990s and from 24.000 to 49.000 ha of forest plantations during the period 2000-2009 depending on availability of resources, including foreign assistance (Gaafar, 2011).

Human activity and drought have had severe impact in Butana area. Akhtar (1994) and Lazim (2003) mentioned that trees and shrubs cover in Butana has changed and become inadequate, and (Mohammed, 2013) reported that the activity of rain fed agriculture has taken more land forests in Butana

According to FAO the term forest plantation include all forests established by planting or seedling in the process of afforestation and reforestation. It related to indigenous and

introduced species (Bredemeier and Achim, 2011). *Acacia mellifera* species is indigenous in Butana, *A.mellifera* and with *A.tortilis* provides great quantities of browse in the Butana region, through provision of fodder for livestock during critical periods when grasses and herb are not available (Lazim, 2003). *Moringa olifera* is considered one of the most useful trees. It is a drought-tolerant grows best in dry-sandy soils, it is adapted to various soil conditions: it can grow in poor soils from pH 4.5 to8. It is grown in areas with a wide range of rainfall, from 25 to up 300 mm/year and up to 1000 of altitude (Hsu et al., 2006; Rashid et al., 2008; FAO-Eco crop, 2009). The objective of this study was to plantation *Acacia millefera* and *Moringa olifera* species and compare performance both trees as reforestation options in Butana area.

Materials and Methods

Study area: The study was carried out in Butana area, lies in the east central part of Sudan between latitude 12º30-16º30N and longitudes 33º35-36º35E; at Sobagh village (15°.3.123 N-034°.78728E) (IFAD, enclosure) and at EL-Adid ELtuwaltoal village (15°.29637 N - 034°.79056E) (farmers' fields). The study area is characterized by low rainfall (302 mm - 280 mm/year). Two forest species were used in this study: *Acacia millefera* (Kitir) for the rapid establishment of woody ground cover, native to Butana, and *Moringa olifera* (Moringa), a fast growing and drought resistant, introduced as exogenous tree species in Butana

Plantation: To establish the seedlings in the nursery, soil mixture of river silt and sand at 1:1 ratio by volume were packed into 10×20 cm polythene bags punctured at the lower 2/3 of the bag. The filled bags were arranged in seedling bed and were flood irrigated in such a way to make sure that water entered the bag through imbibitions this allows smooth wetting of the seeds sown at the top to get moisture without disturbance. Irrigation was done every other day at the beginning then gradually reduced to once and twice weekly. Weeding was done after seedlings emergence to a reasonable height. After four months seedlings were transplanted to field at both sites, the seedlings were grown in a Randomized Complete Block Design (RCBD) with three replications; distance between plants in rows was 1m.

Parameters: The following growth parameters were recorded every month following planting:

Height (cm): was measured from the soil surface to the tip of the main leaves using a ruler.

Diameter at root collar (mm): was measured at five cm from the ground using the Vernier.

Branch number per plant: was done by counting all branches.

Survival percent: was estimated by counting the number of surviving seedlings and dividing it by the initial number of seedlings x 100.

Results and Discussion

Height (cm): A gradual increase was observed in height after 150,180 and 210 days at both sites for Moringa (Figure 1).In *Acacia mellifera* (Figure 2) for the first month the increase in height showed a higher rate at Sobagh. For the second month the increase in height showed the same trend(66.4cm), but here the rate of increase was higher for Sobagh after 180 days (age six month)(77.6cm)than EL-Adid ELtuwal (68.9cm). This could be related to the better soil type found in this area.

Stem diameter (mm): Stem diameter (mm**)** increased gradually with advancement of growth for the first month in field for both tree species. The increase was more pronounced for *Moringa olifera* throughout the period (180 days) (Figure 3), the same observation was shown for *Acacia mellifera* but differences were more pronounced after 180 days, Stem diameter varied from 0.26 to 0.22 mm at Sobagh and EL-Adid ELtuwaltoal respectively (Figure 4).

Number of branches per plant: Effect of site on number of branches per plant showed that for *Moringa olifera*, there was a sharp increase from fifth to sixth months since plantation then there was decline after seventh months. This may be due to strong wind in area which it caused some damage or might be Mmoringa seedlings were not able to survive such competition from other plants (Hurt and Tainton, 1999). These authors reported during the initial stages of growth following germination, woody plants are normally in direct competition with the grasses for resources in the topsoil. Plantation at Sobagh showed a higher number of branches compared to EL-Adid ELtuwal, significant decreases could be detected at fifth ($p< 0.04$) and seventh months of plantation ($p< 0.03$) (Table 1). For *Acacia*, the picture was different where steady increases in number of branches per plant were observed at the two sites with increase of age of the plant, Sobagh recorded less mean new branches, 2.2, than EL-Adid ELtuwaltoal, 2.3, after the first month in field (age five months) (Table 2). This is because that rodents were caused damage on branches (Vander Walt and Le Rich, 1999). They reported rodents, often gather small branches and stalk at multi-stemmed base of *Acacia mellifera* to protect themselves against birds of prey. Generally, it was increase of vegetative growth in this plant and this possibly due to the greater root systems of tree seedlings which it's penetrates the shallow soil layer faster imbibition of moisture and this reflecting as increase in seedling growth. Shallow tree species, like *Acacia mellifera* and *Grewia flave* species, increase in abundance following overgrazing of the grass layer, which suggests that they are favoured by an increase in water availability in the upper soil layer (Skarpe, 1990). Again Sobagh area showed better measurements than EL-Adid ELtuwal. However significant increases could not be detected with the advancement of age of the plant.

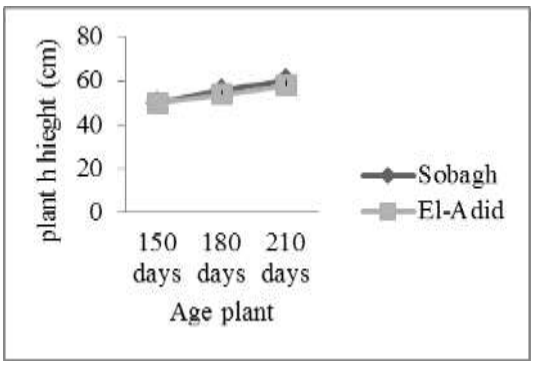


Figure 1.Effect of site on height (cm) of *Moringa olifera* 1st year

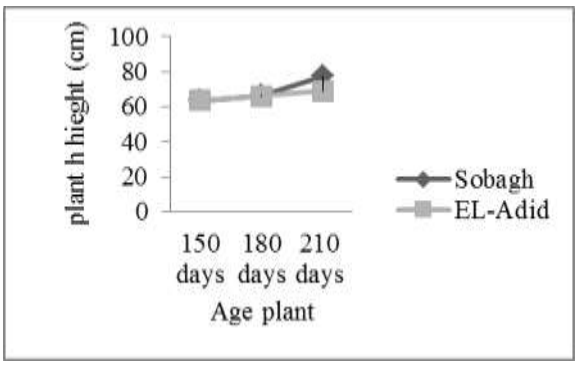


Figure 2. Effect of site on hieght (cm) of *Acacia mellifera* 1st year

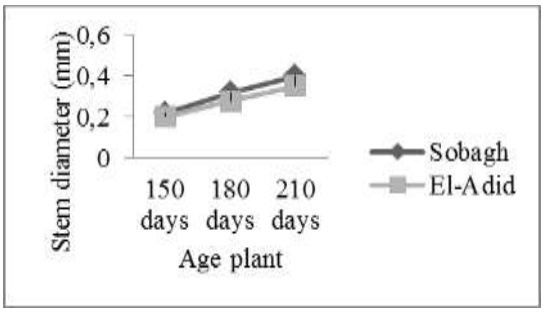


Figure 3 Effect of site on stem diameter of *Moringa olifera*

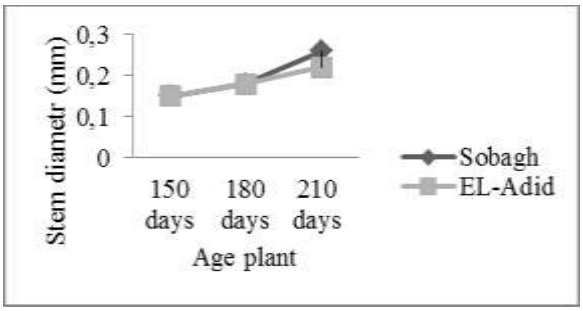


Figure 4Effect of site on stem diameter, of *Acacia mellifera*

Table 1. Effect of site after 5, 6, 7 months of plantation on number of branches of *Moringa olifera*

Site	5th month	6th month	7th month
Sobagh	3.02	5.10	3.31
EL-Adid ELtuwal	3.79	4.24	2.63
Mean	3.14	4.67	2.97
P-Value	0.04	0.06	0.03

Table 2. Effect of site after 5, 6, 7 months of plantation on number of branches of *Acacia mellifera*

Site	5th month	6th month	7th month
Sobagh	2.19	2.7	3.94
EL-Adid ELtuwal	2.47	2.7	3.48
Mean	2.33	2.7	3.71
P-Value	0.23	0.98	0.20

The differences obtained between the two trees could be related to the better adaptation of *Acacia mellifera* to the prevailing conditions at both sites since Acacia is considered as indigenous tree. It could also be observed that Sobagh soil is better than EL-Adid ELtuwal supporting better branches yield. The better plant performance shown by Sobagh site could be attributed to the fact that all measurements were taken inside an enclosure planted yearly with leguminous herbs to be provided as fodder for animals during the dry season. This would have greatly added to the fertility of the soil resulting in better growth.

Survival (%): Survival percent for *Moringa olifera* showed a steady decrease at seventh months of plantation then a sharp decrease at 13 months, woody plant seedlings require sufficient light and water if they are to establish successfully. According to Trollope (1981), favorable water condition is necessary during the establishment period; With Sobagh area shows a better survival percentage (Figure 6). For *Acacia mellifera* slight steady increases were observed (Figure 5). The better performance of *Acacia mellifera* compared to *Moringa olifera* (Figure7) during second year could be attributed to the fact that the former is considered as indigenous species adapted to environmental conditions of Butana area. Similarly, it was recommended that other acacia trees like *A. senegal* (Hashab), *A. nilotica*(Sunt), *A. seyal* (Talh) and *A. mellifera* (Kitir), would constitute the major component in afforestation and reforestation in areas of less than 500mm/annum rainfall.(ELsiddig *et al*., 2008)

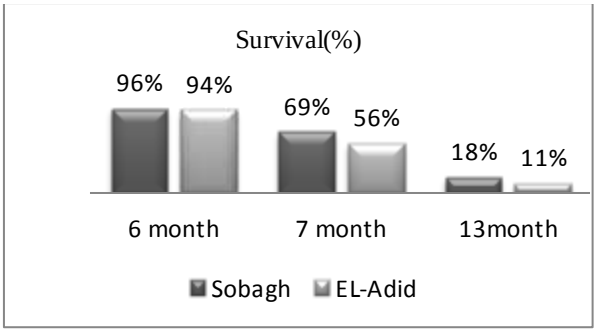


Figure 5. Survival (%) of *Acacia mellifera* at the two sites the during the 1st t year

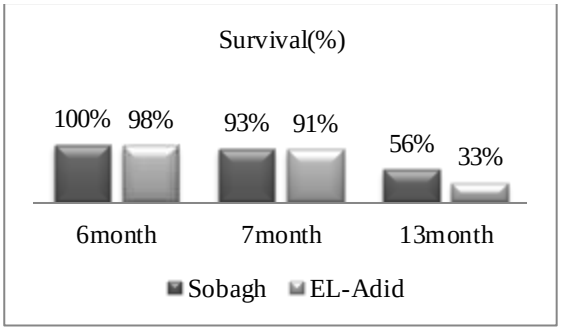


Figure 6. Survival (%) of *Moringa olifera* at the two sites during the 1st year

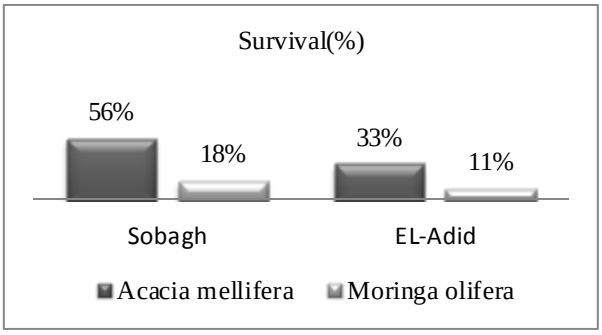


Figure 7. Survival (%) of *Moringa olifera* and *Acacia mellifera* seedlings in two sites during 2nd year

Conclusions

Better establishment of *Acacia mellifera*, it has been adapted to environmental conditions of Butana area than *Moringa olifera*. Perennial browse indigenous trees is recommended to be used in reforestation options, it could improve soil fertility and will provide continuous source of fodder all the year round.

Acknowledgments

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FUNGI LINKED TO DIE-BACK AND DECAY OF NORWAY SPRUCE STANDS IN BOSNIA AND HERZEGOVINA

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Abstract

Norway spruce (*Picea abies*) represents one of the most economically and ecologically important conifer species in Europe, with a long tradition of artificial regeneration and stand establishment. In recent years, spruce die-back has been observed throughout Norway spruce range, including Bosnia and Herzegovina (BiH), where significant die-back of spruce trees has been reported since 2012, both in natural stands and in plantations. The aim of this study was to conduct field investigations, describe symptoms and signs of possible insect or disease problems, and to isolate and morphologically characterize fungi associated with the die-back and decay of Norway spruce trees and logs in BiH. Symptoms and signs observed during these investigations included discoloration of needles and needle drop off, abundant resin flow on the outer bark of the trees, necrotic lesions, insects galleries under the bark, crown die-back, but also death of the entire trees, wood decay of standing trees and logs and the appearance of fungal fruit bodies. Fungi belonging to both Ascomycota and Basidiomycota were found associated with the symptomatic trees and decayed tree logs and these included wood-decay fungi, i.e. *Fomitopsis pinicola*, *Armillaria* spp., *Heterobasidion* spp., Nectriaceae spp., and ophiostomatoid fungi that have developed a symbiotic relationship with bark beetles. We speculate that climate extremes (long periods without rainfall accompanied by high temperatures), insects (primarily bark beetles), and fungi that inhabit physiologically weakened spruce trees might have contributed the observed tree die-back. Proper identification of fungal species involved in the die-back and decay of spruce trees in BiH is crucial for appropriate disease management strategies.

Key words: *Spruce die-back, wood decaying fungi, Nectriaceae, ophiostomatoid fungi, fungus-insect symbiosis.*

Introduction

Norway spruce[*Picea abies* (L.) Karst.] is a dominant species of Boreal and subalpine coniferous forests, stretching from central, through northern and eastern Europe to the Urals, where it merges with Siberian spruce (*Picea obovata* Ledeb.), which is considered a subspecies of *P. abies* (Farjon and Filer, 2013). The altitude and amplitude of this species range from sea level in northern Europe to 2400 m above sea level in the Alps, where it grows in bushy formations (Leuschner and Ellenberg, 2017). In southern Europe, *P. abies* survived the last ice age in several refuges from which the process of colonization of this species later took place in several independent routes, which led to significant genetic variation of spruce in this region (Tsuda et al., 2016; Stojnić et al., 2019). In Bosnia and Herzegovina (BiH), *P. abies* represents one of the most economically important species. The total area of forests in which *P. abies* occurs as the main or secondary species in communities with beech and fir in BiH is 12% (Lojo et al., 2011).

Beginning in the 1980s, *P. abies* forests in the mountainous areas of Central and Southern Europe have begun to show symptoms like crown die-back, needle discolouration, and reduced tree growth. Air pollution is suggested as one of the causes of these phenomena (Jansson, 2013). Moreover, due to *P. abies* ecological requirements for cold and humid climates, but also limited opportunities to adapt to drought and increased CO_2 concentrations, this economically valuable species is expected to be severely affected by global climate change (Linnakoski et al., 2017). Physiologically weakened *P. abies* trees become prone to bark beetle infestations (e.g. *Ips typographus* L., *Pityogenes chalcographus* L., Coleoptera: Scolytinae), and fungal infections (Bugmann, 2014).

The basidiomycete fungus *Heterobasidion annosum* (Fr.) Bref causes root and butt rot of *P. abies* trees throughout the northern hemisphere, leading to large economic losses caused by reductions in both tree growth and wood quality (Keča, 2008; Garbelotto and Gonthier 2013; Zlatković et al. 2018). Ophiostomatoid fungi associated with insects belonging to the genera *Ips*, *Dendroctonus* and *Scolytus* have been identified as the main pathogens of *P. abies* trees in Europe (Linnakoski, 2011). Moreover, in the last ten years, the damage caused by fungi belonging to Nectriaceae has intensified, and these fungi are causing necrotic lesions, cankers,, abundant resin production, and die-back of older trees (Lilja et al., 2012; Lombard et al., 2015). *Fomitopsis pinicola* (Sw.) P. Karst. is a common fungus in boreal and temperate *P. abies* forests of the northern hemisphere and it occurs as a saprophyte on dead wood and as a tree pathogen of various hardwood and softwood trees, causing brown crumbly rot (Shah et al., 2018; Wu et al., 2018; Zlatković et al. 2018).

In BiH, extreme drought recorded in 2012 has been suggested as the cause of a mass die-back of *P. abies* trees in certain areas of the country. Mistakes in forest management practices and the lack of appropriate economic measures have negatively affected the stability of the forest ecosystems in this country, leading to repeated outbreaks of fungi and insects (Usčuplić and Dautbašić, 1998; Treštić et al. 2007.; Dautbašić et al., 2014; Stanivuković and Vasiljević, 2018; Zahirović et al. 2019).

Since most previous studies focused only on the inventory of a potentially harmful fungi preliminary identified in the field based on disease symptoms and fruit bodies, and that little attention was paid to fungi causing wood rot of fallen wood and logs, the aim of this study was to investigate the current extent of *P. abies* die-back, describe the symptoms observed in the field, and to isolate and morphologically characterize fungi associated with the die-back and decay of *P. abies* trees and logs in natural stands and plantations in BiH.

Material and Methods

The fieldwork was performed during the vegetation seasons 2018 and 2019 in BiH, in the entire natural range of *P. abies*, as well as in *P. abies* plantations, in areas where intensive die-back of this species was recorded in previous years (Cadastre PFE, 2016; IFM, 2019). In the forest area managed by the Public Forestry Enterprise "Šume Republike Srpske- Forests of the Republic of Srpska" a.d. Sokolac, the research was performed in several forest enterprises, namely: "Romanija" Sokolac, "Sjemeć" Rogatica, "Visocnik" Han Pijesak, "Čemernica" Kneževo, "Klekovača-Potoci" East Drvar and "Ostrelj-Drinic" Drinic. Samples were also collected in the National Park "Sutjeska" Foca and "Drina" Srebrenica. Moreover, research was conducted in Bosnia and Herzegovina, in the area managed by the Forest Business Association of Zenica - Doboj Canton, management unit Forestry Olovo [working unit Dubostica] and Zavidovici (Vozuca); in the area managed by the Forest company "Srednjobosanske šume", in the areas of Busovača Forestry (Busovača) and Travnik Forestry (Vlašić) and in the area of the Cantonal Public Enterprise for State Forest Management "Sarajevo Forests", Srednje (Gajevi).

Depending on the stage of the die-back of *P. abies* trees, one to three experimental plots were set up at the above-mentioned localities. In each experimental plot, samples at chest height were taken from ten symptomatic spruce trees (die-back, abundant resin flow, wood decay, necrotic lesions), using a Presler drill. The coordinates of each tree from which the samples were taken were recorded using a GPS device. The collected wood samples were packed in 8 mm diameter straws, transferred to the laboratory for forest pathology of the Institute of Lowland Forestry and Environment (ILFE) in Novi Sad and stored at 4°C until further processing. The field experiments were conducted at altitudes that ranged from 530 to 1620 m.

The collected wood was cut into 4x4 mm fragments, and then fragments containing necrotic and healthy tissue as well as those showing bluish discolouration were selected for further analyses. Ten symptomatic wood fragments per tree were selected and these were arranged in two Petri dishes (five fragments per Petri dish). The fragments were then surface sterilized by immersion in 70% ethanol (1 min), washed in sterile distilled water, dried on sterile paper towels, held shortly under a flame and placed onto 2% malt extract agar medium acidified with lactic acid (AMEA, Zlatković et al., 2016). In addition to fragments taken from *P. abies* trees, fungal fruiting bodies (basidiocarps), parts of mycelium and rhizomorphs of fungi residing in *Armillaria* were also collected. The necrotic lesions were cut into 4x4 mm fragments so that each fragment covered pieces of dead and seemingly healthy wood, and then placed on AMEA as previously described.

Moreover, bark beetles were collected from pheromone traps of the TYSON or ECOTRAP type (with pheromones of the *IT ecolure and PC ecolure type*), placed in paper bags and transferred to the laboratory. The insects were identified using a stereomicroscope Olympus (model SZX10, Tokyo, Japan). This was done based on the size of the insects, their wing covers, and "teeth". From each sampled site, 50 individuals of each identified beetle were surface sterilized by immersion in a solution of 0.05% Tween 80 (Fisher BioReagents®, Pittsburgh, Pennsylvania, US) (1 min), washed in sterile distilled water and dried on sterile paper towels. After drying, the insects were divided into two parts, the head-thorax and abdomen, and these parts were placed on malt extract agar (MEA) and AMEA media with sterile tweezers: half of the insects were placed on 2% AMEA medium, and the other half of the insects were placed on Ophiostoma selective 2% MEA medium supplemented with the streptomycin (0.05%, HIMedia Laboratories Pvt. Ltd, Mumbai, India) and cycloheximide (0.04%, Fisher BioReagents®, Pittsburgh, Pennsylvania, US) (Wingfield et al., 1993).

Results and Discussion

In total, 1003 fungal isolates were obtained from wood fragments, bark beetles, necrotic lesions, spore prints from basidiocarps, and parts of mycelium found in maternal tunnel and larval tunnels of bark beetles. These could be divided into five morphological groups, i.e. Ophiostomatoid fungi (94 isolates), Nectriaceae spp. (32 isolates), *Heterobasidion* spp. (12 isolates), *Armillaria* spp. (11 isolates) and *Fomitopsis pinicola* (seven isolates). Moreover, one isolate was preliminarily identified as *Xylaria* sp. Also, fungi belonging to genera *Alternaria* and *Aureobasidion* were isolated in this study, but they were excluded from further research due to their well-known endophytic nature in forest trees (Andrews et al. 2002).

Collected bark beetles were identified as *Pythiogenes chalcographus* L. - six-toothed spruce bark beetle, *Ips typographus* - eight-toothed spruce bark beetle and *Polygraphus poligraphus* L.- small spruce bark beetle.

Morphological group 1 - Ophiostomatoid fungi

Based on morphological characteristics like mycelial growth pattern and colour, rapid production of fruiting bodies in culture in the dark, the morphology of fruiting bodies (perithecia with characteristic long black necks, groups of black conidiophores bearing balls of sticky spores on the top - coremia), shape and dimensions of spores, 96 isolates obtained in this study were preliminarily identified as ophiostomatoid fungi. From these, 34 isolates were isolated from wood samples, 24 isolates were isolated from *P. chalcographus*, 32 isolates were obtained from *I. typographus*, 4 isolates were obtained from white mycelium present in the maternal tunnel and larval tunnels, while 2 isolates originated from mites phoretic on bark beetles. The isolates were further divided into nine morphological subgroups and representatives of each of these subgroups will be further identified up to the species level using molecular phylogenetic analyses. Initial morphological examinations revealed that the colours of the mycelium of the obtained isolates varied from white, yellowish, to brown, grey and black, whereas some of the colonies were wholly on the surface. The cultures were characterized by rapid growth at 22°C. Sexual fruit bodies known as perithecia were brown to black, with a long straight or slightly curved neck (measuring 340-390 µm in length). However, asexual fruit bodes were also often observed and these consisted of hyphae aggregated into dark stalks known as synnemata. Conidia were hyaline, aseptate, oval or fusiform in size (3.5-)-4-5.5(-6.8) x 1-2 µm.

Ophiostomatoid fungi represent an interesting fungal group that includes fungi from the genera Ceratocystis (order Microascales) and Ophiostoma, Grosmannia, and Ceratocystiopsis (order Ophiostomatales) (Upadhyay, 1993; Linnakoski, 2011). Some species are causing bluish discoloration of sapwood of coniferous trees and sawn timber (Wingfield et al., 1993; Jacobs and Wingfield, 2001), while others, like *Ophiostoma ulmi* (Buisman) Melin & Nannf., *Ophiostoma himal-ulmi* Brasier & Mehrotra and *Ophiostoma novo-ulmi* Brasier are dangerous pathogens causing Dutch elm disease (Brasier, 1991).

In this study, ophiostomatoid fungi were isolated from symptomatic wood, bark beetles (*P. chalcographus* and *I. typographus)*, white mycelium that was present in the maternal tunnel and larval tunnels and from mites. Preliminary results indicate that a huge diversity of these fungi exist in this area. Moreover, preliminary morphological characterization indicated that most isolates likely belong to the *Ophiostoma piceae* species complex. This is expected as fungi belonging to the *O. piceae* species complex are well-known pathogens of *P. abies* trees (Linnakoski et al., 2010; Repe, 2014). However, it is necessary to morphologically characterize all obtained isolates and to identify representatives of each morphological group up to the species level using molecular phylogenetic methods.

Morphological group 2 - Nectriaceae spp.

Based on the mycelial growth pattern and growth rate, morphological characteristics of the fruiting bodies formed in culture, shape, and dimensions of spores, 32 isolates obtained in this study were classified into the morphological group Nectriaceae. Of this number, 19 isolates were obtained by isolation from necrotic lesions, while 13 isolates were obtained by isolation from wood fragments. The cultures were mostly cotton-like and white at the beginning of the growth, later acquiring an orange-red hue, and their growth at 22 °C was relatively slow. Most cultures formed cream-coloured sporodochia that contained fusiform macroconidia and oval microconidia measuring (3.5-)-4-6(-6.5) x 2-2.5 µm. The most important Nectriaceae spp. that occur in Europe include *Corinectria fuckeliana* (C. Booth) C. González & P. Chaverri which is a pathogen of spruce (Dick and Crane, 2009; Lilja et al., 2012; Pettersson et al., 2018) and *Neonectria neomacrospora* (C. Booth & Samuels) Mantiri & Samuels which is a pathogen of fir, but can also be found on spruce (Pettersson et al., 2016).

Given that the isolates were obtained from necrotic spruce tissue and that the morphological characteristics of most isolates were such that microconidia in sporodochia were numerous and conidia were most often not present, we can speculate that most of the isolates obtained represent *C. fuckeliana*. However, detailed molecular analyzes are necessary to provide adequate information on the identity of the collected isolates.

Morphological group 3 - *Heterobasidion* spp.

Based on the mycelial growth pattern and growth rate of the cultures, conidiophores formed in culture, shape, and dimensions of basidiospores, 12 isolates from this study were classified in the morphological group *Heterobasidion* spp. All 12 isolates were obtained by isolation from wood fragments.

Colonies of *Heterobasidion* spp. were characterized by rapid growth, they were white, and after ten days of growth cotton-like and occasionally with yellowish sectors, but one culture after two weeks of growth in the dark at 22°C got a yellowish tint on the entire surface of the colony, and was morphologically visibly different from the others. Moreover, conidiophores with conidia could be seen in the cultures under the stereomicroscope. The basidia were with four sterigmates at the top. Basidiospores were hyaline, ovoid to ellipsoidal, pointed at the end, measuring 4-7.5 x 3.4-4.5 µm. The hyphae were simple septate, mostly without clamp connections. Fruiting bodies (basidiocarps) were not found.

Heterobasidion annosum (Fr.) Bref *sensu lato* complex of species includes some of the most important coniferous wood decay fungi, found throughout Eurasia and North America (Garbelotto and Gonthier 2013). Symptoms caused by *Heterobasidion* species vary depending on the identity of the pathogen, tree species, stand age, soil type, local climate, and possible air pollution (Garbelotto and Gonthier 2013). The process of tree die-back is initiated by the appearance of a stress factor (most often drought), but the host genotype also plays a significant role in the development of diseases caused by these fungi by limiting or preventing the spread of the disease through root contacts (Piri and Valkonen, 2013; Keriö et al., 2014).

Considering the fact that two mycelial types were obtained in this study, and that Zahirović et al. (2019) showed that both, *H. annosum* and *Heterobasidion parviporum* Niemela & Korhonen are present in BiH, we can speculate that the two morphologically different *Heterobasidion*-like isolates represent the two species but molecular phylogenetic analyzes are necessary to confirm this suggestion.

Morphological group 4 - *Armillaria* spp.

Based on the presence of black rhizomorphs and white fan-shaped mycelium under the bark of the infected trees, the spore print of fruiting bodies (mushrooms), as well as the shape and dimensions of basidiospores, 11 isolates obtained in this study were classified into the morphological group *Armillaria* spp. Colonies of these isolates showed a rather slow growth on MEA at room temperature and were whitish with a black margin or black with a white margin, occasionally with rhizomorphs that spread below the mycelium. Fruiting bodies (mushrooms) grew densely in groups (bouquets) at the base of the sampled symptomatic trees. The cap of the mushroom was light brown in colour with dark brown scales, and it was moist and sticky to the touch, with a white slightly descending gills. The stem was also whitish, hard and with a ring. Basidiospores were ellipsoidal, hyaline, measuring 6.11-10.63 x 4.43-5.76 µm.

The wide host range, virulence, and production of rhizomorphs that spread through the soil allow *Armillaria* species to survive for decades and even centuries in the forest and cause damage (Morrison, 2004). In this study, white fan-shaped mycelium, and black rhizomorphs characteristic of *Armillaria* species were visible after the bark of symptomatic *P. abies* trees was removed. Moreover, mushrooms with light brown caps with dark scales, and with stems

bearing rings, white spore print and ellipsoidal basidiospores, that morphologically correspond to *Armillaria* spp., were observed at the base of the infected trees. Zahirović et al. (2019) found *Armillaria cepistipes* Velen. and *Armillaria ostoyae* (Romagnesi) Herink on *P. abies* trees in BiH, whereas Keča et al. (2009) identified *Armillaria gallica* Marxm., *A. cepistipes* and *A. ostoyae* in Serbia. However molecular phylogenetic analyses are necessary to reveal the identity of the *Armillaria*-like isolates obtained in this study.

Morphological group 5 – *Fomitopsis pinicola*

Based on the morphological characteristics of the fruiting bodies (basidiocarps), the type of the wood rot, the mycelial growth pattern and growth rate and the dimensions and shape of the basidiospores, seven isolates obtained in this study were classified into a morphological group *Fomitopsis pinicola*. Colonies of these isolates were white and woolly, growing rapidly at 22^{o}C in the dark. The basidia were with four sterigmates at the top. Basidiospores were cylindrical, hyaline, with a pointed tip, measuring 6.5-8.7 x 4.0-4.5 µm. Fruiting bodies (basidiocarps) were perennial, woody, reddish-brown on the upper side, with a white or yellowish edge. Pores were white to cream-coloured. The fact that the fungus has not been isolated from still living symptomatic trees indicates that *F. pinicola* is a final destructor of *P. abies* wood in *P. abies* stands affected by die-back in BiH. However, this hypothesis requires further studies.

Conclusions

P. chalcographus - six-toothed spruce bark beetle, *I. typographus* - eight-toothed spruce bark beetle and *P. poligraphus* - small spruce bark beetle was found in *P. abies* stands in BiH.

F. pinicola, *Armillaria* spp., *Heterobasidion* spp. and *Xylaria* sp., Nectriaceae spp. and ophiostomatoid fungi were isolated in this study but these fungi were identified using morphological characteristics only.

Molecular phylogenetic analyses are necessary to identify isolated fungi up to the species level. This is important because adequate identification of potentially harmful fungi represents the first step leading to reducing existing and preventing new damages in already vulnerable Norway spruce stands.

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